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INFLUENCE OF THREE PLANT EXTRACTS ON *FUSARIUM OXYSPORUM* F. SP. *CICERIS* MYCELIUM GROWTH

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Abstract: Three plant extracts *viz.* bulbs of *Allium sativum* L. (*Liliaceae*), seeds of *Annona squamosa* L. (*Annonaceae*) and leaves of *Vitex negundo* L. (*Verbenaceae*) were evaluated against cowpea wilt pathogen, *Fusarium oxysporum* f. sp. *ciceris* by mycelial dry weight method under laboratory condtions. The mean mycelium dry weights of *F. oxysporum* of methanol and benzene extracts of *A. sativum* obtained from 125 g of crused dry plant material (bulbs) were 0.0113 and 0.0174 mg, respectively. This was followed by methanol and petroleum ether extracts of *A. squamosa* (0.2396 and 0.2381 mg). They effectively controlled mycelial growth of cowpea wilt pathogen, however *V. negundo* extracts did not cause any significant mycelium growth inhibition when compared to other plant extracts tested. Among the three plant extracts, methanol extracts of *A. sativum* bulbs could possibly be used for controlling *F. oxysporum.*

Key words: Cowpea wilt pathogen, *Fusarium oxysporum*, *Allium sativum, Annona squamosa, Vitex negundo,* growth inhibition

INTRODUCTION

Cowpea is an important pulse crop in India and is cultivated on more than nine million hectares. But its production is limited by wilt and rot disease caused by *Fusarium oxysporum* f. sp. *ciceris* (Rangeswaran and Prasad 2000). Even though synthetic fungicides are available and also used to control this disease, their indiscriminate use caused various environmental hazards. Hence it is necessary to develop ecologically safe, effective and economically payable method of disease management (Horst et al. 1992). The use of plant extracts has long been recognized as an area of investigation (Wilson and Wismeiwaki 1994; Srivastava and Lal 1997; Chatterjee 2000) against various fungal disease control. The present study deals with the evaluation of control efficacy of benzene, methanol, petroleum ether and water extracts of seeds of *Annona squamosa* L.

(*Annonaceae*), and leaves of *Vitex negundo* L. (*Verbenaceae*) and bulb extract of *Allivum sativum* L. (*Liliaceae*) against *Fusarium oxysporum* f. sp. *ciceris* under *in vitro* conditions.

MATERIALS AND METHODS

Preparation of plant extracts

Fresh leaves of *V. negundo*, seeds of *A. squamosa* and bulbs of *A. sativum* were washed with sterile distilled water, and shade dried for two weeks. After drying, they were crushed using pestle and mortar. Known weight (125 g) of crushed plant material was extracted in saxlet apparatus using either benzene, petroleum ether, methanol and water (40–60°C). The residues of solvent were removed under reduced pressure. Then the distillated and crude extracts were dried in a vacuum desicator and the final residue was collected, stored in a refrigirator at 4°C for further use.

Isolation of fungal pathogen

Infected by *F. oxysporum* f. sp. *ciceris* cowpea leaves were collected from Crop Protection Research Centre farm, St. Xavier's College, Palayamkottai, Tamil Nadu, India and brought to laboratory in a sterile container and used for further isolation of fungal pathogens. The infected leaves were cut into small pieces (0.5 x 0.5 cm), and transferred into petri plates containing 0.1% $HgCl_2$ solution for 5 minutes. Then they were rinsed with distilled water, transferred into the sterile sabouraud agar plates and incubated at room temperature. After 72 hours the isolated pathogens were stored in sabouraud agar slants for further analysis.

Fungicide efficacy evaluation

Five mm disc of 72 hours old *F. oxysporum* was inoculated into sterile sabouraud broth (neopeptone = 10 g; dextrose 40 g; distilled water 1000 ml and filter sterilized chloramphenicol (1%) = 10 µl). The plant extract residue was dissolved with a respective solvent and its activity was tested. 500 µl of plant extract (0.05%) was added into seeded sabouraud broth and incubated at room temperature for 10 days. Controls were amended with respective solvents. Three replications were used separately for each solvent extract. After 10 days, the mycelial mat was taken with sterile spatula, placed in sterile dishes containing filter paper. The initial weight of the paper was recorded. The Petri dishes were kept in hot air oven at 50°C for 30 minutes and the final weight of the fungal mat along with the filter paper was recorded immediately. The difference between the final and initial weight was considered as dry weight of mycelium.

Statistical analysis

The mean data were analysed by Duncan's Multiple Range Test (DMRT) (Duncan 1955) and significance of results was expressed at 5% level.

RESULTS

Among the three plants tested, all the solvent extracts of *A. sativum* were found highly effective in reducing the mycelial growth of *F. oxysporum*. For instance, the mean weight of mycelial growth of *F. oxysporum* in methanol and petroleum ether extracts of *A. sativum* was 0.0113 and 0.0161 mg, respectively. When compared the

A. sativum to other plants, results were highly significant ($p < 0.05$). The benzene and methanol extracts of *A. squamosa* also inhibited the fungal growth to some extant (Table 1). The mycelium mean weights of petroleum ether and methanol extracts were found to be 0.2381 and 0.2396 mg, respectively. *V. negundo* did not cause any significant reduction of mycelial growth when compared to other plants. The order of recorded activity was: *A. sativum* > *A. squomosa* > *V. negundo.*

Table 1. Influence of different plant extracts on the mycelium growth of *Fusarium oxysporum*

Plant	Plant parts used	Mycelial dry weight [mg]			
		Benzene	Methanol	Petroleumether	Water
Vitex negundo	Leaves	0.3101a	0.3010 a	0.3012 a	0.3091 a
Annona squamosa	Seeds	0.2431 b	0.2396 b	0.2381 b	0.3114 ab
Allium sativum	Bulbs	0.0174c	0.0113 c	0.0161 c	0.1890 c
Control	Solvents	0.4121	0. 4041	0.4132	0.4123

Significant at 5% level by Duncan's Multiple Range Test

DISCUSSION

Previously 31 plants belonging to *Astraceae* family (Rai and Acharya 1999) were tested against the cowpea wilt pathogen *F. oxysporum* f. sp. *ciceris.* Ozer et al. (2003) evaluated the pectolytic impact of *Allium cepa* 'Akgun 12' against two *F. oxysporum* isolates FOC6 and FOC8. In addition *A. sativum* was also known to act as anti-bacterial, anti-inflammatory and anti-hepatotoxic and this is due to the bioactive components like bialy/disulphide oxide (alicin) (Hikino et al. 1986). Allylmercaptocysteine and 5 namylmercaptocysteine of Anomaine is the bioactive compound present in *Annona squomosa* seeds which has anti-bacterial, anti-fungal and anti-insecticidal properties (Oliver-Bever 1986). Though *V. negundo* has been used for controlling expectorant leprosy treatment, cancer therapy and ear disease (Panigraphi and Alaka Sahu 2000), the present study showed that it did not cause any significant growth inhibition of *F. oxysporum*. Further studies on control the disease in field conditions with plant extracts are recommended.

CONCLUSIONS

Of all the tested plant extracts, methanol and benzene extracts of *A. sativum* showed the most effective control efficacy against the cowpea wilt pathogen; *F. oxysporum* f. sp. *ciceris*.

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POLISH SUMMARY

WPŁYW TRZECH EKSTRAKTÓW ROŚLINNYCH NA WZROST GRZYBNI GATUNKU *FUSARIUM OXYSPORUM* F. SP. *CICERIS*

W warunkach badano laboratoryjnych wpływ trzech ekstraktów roślinnych otrzymanych z cebul *Allium sativum* L. (*Liliaceace*), nasion *Annona squamosa* L. *(Annonacease)* i liści *Vitex negundo* L. (*Verbenaceae*) na wzrost grzybni gatunku *Fusarium oxysporum* f. sp. *ciceris*. Średnia sucha waga grzybni po zastosowaniu ekstraktów z *A. sativum* w metanolu i benzenie, uzyskanych z 125 g suchego materiału roślinnego wynosiła odpowiednio 0.0113 oraz 0.0174 mg. Sucha waga grzybni po zastosowaniu ekstraktów w metanolu oraz benzynie z nasion *A. squamosa* wynosiła 0.2396 i 0.2381 mg. Ekstrakty te skutecznie ograniczały wzrost grzybni gatunku *F. oxysporum* f. sp. *ciceris*. Ekstrakty otrzymane z *V. negundo* nie wykazały istotnego działania w porównaniu do pozostałych ekstraktów. Spośród wszystkich testowanych ekstraktów, ekstrakt w metanolu otrzymany z *A. sativum* mógłby być używany do zwalczania *F. oxysporum* f. sp. *ciceris*.