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IN-VITRO ASSAY OF SOME PLANT EXTRACTS AGAINST *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* CAUSAL AGENT OF TOMATO WILT

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Abstract: The effect of crude extracts of neem (*Azadirachta indica*) leaf, neem seed and garlic (*Allium sativum*) at concentrations ranging from 5% to 30% of the material in 100 ml of Potato Dextrose Agar on mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici* was assessed. All the extracts inhibited mycellial growth at various levels. Dry neem seed extract gavel 100% inhibition of mycelial growth. Fresh neem leaf extract reduced mycelial growth with increasing concentration while in garlic there were no differences in growth inhibition among the various concentrations used. However garlic extracts decreased sporulation with increasing concentration and cultures grown on extract amended agar plates remained viable.

Key words: plant extracts, *Fusarium*, tomato, wilt, Nigeria

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is an important disease of tomato in the Nigeria savanna, where it is often found in a synergistic relationship with *Meloidogyne incognita.* Recent advances in nematode and *Fusarium* wilt control have been achieved through the application of botanical products and antagonistic/non-pathogenic microorganisms. Crude aqueous extracts of various plants have exhibited some levels of antifungal activity (Awuah 1989; Hussani and Deeni 1991). Such extracts contain active compounds that are biodegradable and are selective in their toxicity. *In vitro* and pot trials of some plant products, such as neem (*Azadirachta indica*), have shown nematicidal properties (Agbenin et al. 2005).

The use of plant products for control of *Fusarium* wilt in crops is rather limited, the effect of neem products have been reported to have significant controlling effects on some other fungi, for example on conidial germination of *Sclerospora sacchari* isolates

from maize (Poswal and Akpa 1991) and mycelial growth of *Colletotrichum gloeosporoides* isolats from pawpaw (Shimfe 1984) This present study focuses on the effects of neem and garlic plants on the control of *F. oxysporum* f. sp. *lycopersici* in-vitro.

MATERIALS AND METHODS

Collection and preparation of plant material

Neem leaves and seeds were collected from neem trees within Zaria. Neem seeds were air dried. Garlic bulbs were purchased from local markets in Zaria. Samples of neem leaves weighing 10, 20 and 30 g were surface sterilized with 1.0% sodium hypochlorite (NaOCl) and ground in a mortar. The pounded leaves were transferred into a beaker and 10 ml of sterile distilled water was added. The beakers were covered with sterile aluminium foil and allowed to stand on a laboratory bench for four hours. The neem mixtures were filtered through a fine mesh. Similarly dry neem seeds pounded into powder in a mortar were used instead of leaves. However, 15 ml of sterile water was added and not 10 ml as for fresh neem leaves.

Samples of fresh garlic bulbs weighing 5, 10 and 20 g were surface sterilized with 1.0% sodium hypochlorite (NaOCl) and ground in a mortar. The pounded garlic was transferred into a beaker and 10 ml of autoclaved sterile water was added. The beakers were covered with sterile aluminium foil and allowed to stand on laboratory bench for four hours. The garlic mixture was filtered through a fine mesh. All filtrates from both neem and garlic were stored in a refrigerator at 4°C until required.

Efficacy of neem products

Filtrates of varying concentrations of neem seeds and leaf extract were transferred into separate flasks containing 100 ml of autoclaved potato dextrose agar and streptomycin (PDAS). The mixture in the flask was gently shaken to mix and poured into sterile plates. Plates were allowed to remain on laboratory bench for 24 hours.

One vertical and a horizontal line were drawn under each plate. A 5-day old inoculum of *F. oxysporum* f. sp. *lycopersici* was applied at the intersection of the vertical and horizontal lines drawn under the plate. Growth records of *Fusarium* were taken at 24 hourly intervals for three days for neem leaves and for 12 days for seeds beginning at 24 hours after inoculation.

Efficacy of garlic products

Filtrates of varying concentrations of garlic extract were transferred into separate flasks containing 100 ml of autoclaved potato dextrose agar and streptomycin. The mixture in the flask was gently shaken to mix and poured into sterile plates. Plates were allowed to remain on laboratory bench for 24 hours.

One vertical and a horizontal line were drawn under each plate. A 5-day old inoculum of *F. oxysporum* f. sp. *lycopersici* was applied at the intersection of the vertical and horizontal lines drawn under the plate. Growth records of *Fusarium* were taken at 24 hourly intervals for 12 days beginning at 24 hours after inoculation.

Viability of fungal spores after treatment

Viability of spores from agar plates treated with either neem or garlic extracts was tested. To harvest spores 10 ml of sterile water were poured over the plate. Sterile rod

was gently swapped over it and the mixture poured into a beaker. Three-week old seedlings of tomato variety Roma VF were uprooted from the nursery. Roots were gently washed to remove attached soil and dipped for 2 minutes in the inoculum. Check plants were dipped into sterile water for 2 minutes. Plants were transplanted into pots containing sterile sandy-loam soil. Thereafter, 5 ml of inoculum or sterile water were poured over root of inoculated control plants in the planting hole, respectively. Each treatment was replicated three times and the pots arranged in a completely randomized block design on the screenhouse bench. Plants were observed for symptoms of wilt, four weeks after inoculation. The experiment was repeated twice.

Statistical analysis

The effect of concentrations of the neem leaf and garlic bulb extracts on the growth of *F*. o*xysporum* f.sp. *lycopersici* on PDAS media was evaluated by a one-way analysis of variance (ANOVA). Mean differences between treatments or concentration levels of the extracts were separated by Fisher's least significant difference (LSD) at 5% probability level. The ANOVA was performed with STATVIEW statistical package written for Apple Macintosh PC (Abacus Concepts 1987).

RESULTS AND DISCUSSION

Dry neem seed extract completely inhibited mycelial growth of *F. oxysporum* at all concentrations while extracts of fresh neem leaves reduced mycelial growth with increasing concentrations (Fig. 1). Garlic bulb extracts inhibited mycelial growth by 15.4% as related to the control, but there were no significant differences in growth inhibition among the three concentration levels (Fig. 2). No spores were observed on agar plates with incorporated neem seed extract.

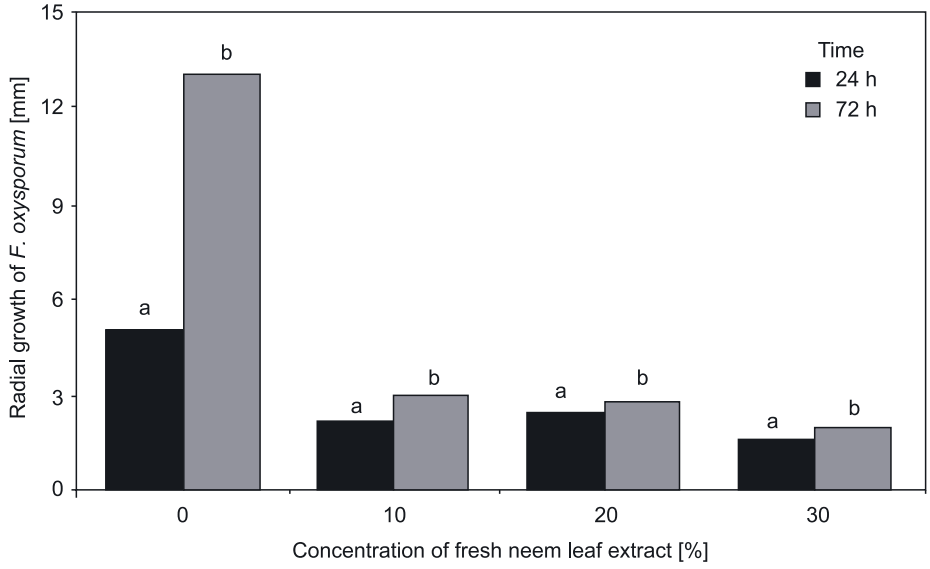

Fig. 1. Effect of fresh neem leaf extract on the growth of *F. oxysporum* on PDAS in 8.5 cm diameter agar plate at 24 and 72 hours. For each time period, bars bearing the same letter are not statistically significant at 5% probability

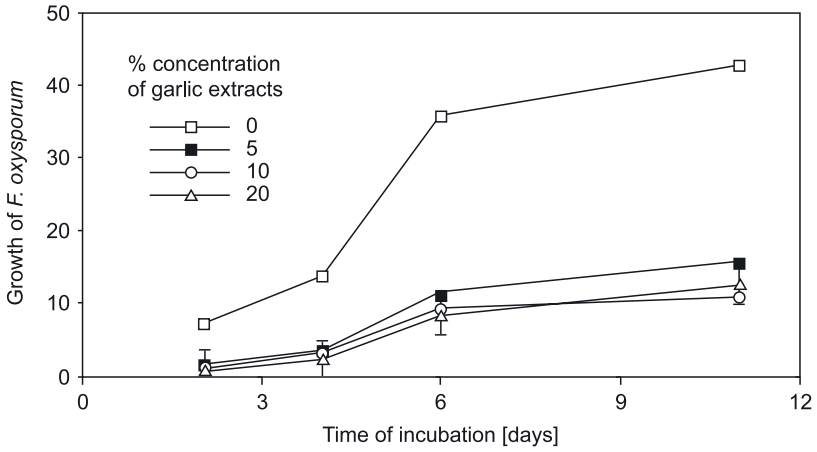

Fig. 2. Effect of garlic bulb extract on the growth of *F. oxysporum* on PDAS in 8.5 cm diameter agar plate

Spores washed from culture of *F. oxysporum* f. sp. *lycopersici* grown on agar plates with incorporated garlic extract remained viable. Young seedlings exposed to these spores by root dipping became infected with wilt causal agent.

Our results agree with those obtained by Olufolaji (1999) on wet rot disease of Amaranthus sp. and *Choanephora cucurbitarum* using neem root bark and fruit extracts. Similarly, Shimfe (1984) observed inhibition of mycelial development of *Colletotrichum gleosporiodes* by extracts of neem leaves and fruit.

Inhibitory activities of plant extracts vary with the plant part used. This explains the difference in toxicity to the fungus exhibited by the dry neem seed and fresh leaf extracts (Gourinath and Manocharachary 1991). *Acorus calamus* completely inhibited the growth of *Botryodiplodia obromae* (Sardsurd et al., 1995), while *Eupatorium cannabinum* showed complete toxicity against *Pythium debaryanum*, *Fusarium oxysporum, Rhizoctonia solani* and *Sclerotium rolfsii*. Although the neem tree has been known to be useful in soil enrichment and for insect, pest and to some extent disease control, its potential for the control of soil-borne diseases especially, *Fusarium* wilt has not been fully exploited. Our results show clearly the potential of neem products for control of *Fusarium* wilt in tomato. Stored samples of garlic powder, *Chrysanthemum, Hedra helix* and *Anagalis arvensis* have also shown persistence in fungitoxicity (Kumar and Tripathi 1991). Some reports indicated that extracts of garlic inhibited tomato mosaic virus when mixed with the virus *in vitro* (Othman et al. 1991).

Garlic extracts reduced mycelial growth of the fungus, but it was not as promising as neem products. However, spores formed remained viable. Spore count was reduced with increasing concentration of extract. Control had the highest spore count of 1.56 x 10^7/ml, while 20% concentration showed the lowest count of 0.06 x 10^7/ml. There was no difference between spore count at 5% and at 10% (0.3 x 10^7/ml). The absence of significant differences among the three concentration levels of garlic extract implies that any of the tested concentration levels would give a similar level of control. Since integrated pest management advocates the use of minimal dosages of chemicals (Hill and Waller 1982), garlic bulb at 5% concentration giving 35% inhibition over control may be useful. This would be appropriate in combination with other control options. However, the reduction in fungitoxicity over time, as shown

by the increase in mycelial growth of the fungus between 6–12 days of incubation on PDA media, suggests that the effect of the extract under field conditions would probably wear off within a very short time span. This would imply that more than one application of the treatment would be required before harvest. This coupled with the high cost of garlic makes it unsuitable as a fungicide. On the other hand, neem trees are abundant in the Nigeria savanna and can easily be procured for their potential fungicidal activity. There is a need to do additional work on the fungicidal effect of neem.

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POLISH SUMMARY

AKTYWNOŚĆ IN VITRO EKTRAKTÓW Z NIEKTÓRYCH ROŚLIN PRZECIWKO *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*, SPRAWCY UWIĄDU POMIDORÓW

Badano działanie surowych ekstraktów z liści i nasion *Azadirachta indica* oraz z ząbków czosnku (*Allium sativum*), dodawanych do pożywki ziemniaczanej z glukozą (PDA) w stężeniach od 5% do 30%, a na wzrost grzybni *Fusarium oxysporum* f. sp. *lycopersici*. Wszystkie badane ekstrakty inhibowały w zróżnicowanym stopniu wzrost grzybni tego patogena. Ekstrakt z suchych nasion *A indica* inhibował wzrost grzybni w 100 procentach. Ekstrakt ze świeżych liści tej rośliny ograniczał wzrost grzybni proporcjonalnie do stężenia w pożywce, natomiast w przypadku ekstrak-

tu z czosnku nie stwierdzono różnic w stopniu inhibicji grzybni w uzależnieniu od stężenia.

Wyciąg z czosnku ograniczał natomiast zarodnikowanie patogena proporcjonalnie do stężenia w pożywce, a kultury rosnące na płytkach agarowych z dodatkiem tego wyciągu zachowywały żywotność.