

## **Antifungal properties of leaf extract of neem and tobacco on three fungal pathogens of tomato (*Lycopersicon Esculentum* Mill)**

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### **ABSTRACT**

Laboratory studies were carried out to isolate, identify and control the fungi associated with fruit rot of tomato (*Lycopersicon esculentum*). *Aspergillus viridae*, *Penicillium digitatum* and *Rhizopus sp* were isolated from samples collected from Lokoja, Kabba, Okene, Anyigba and Ankpa, all in Kogi State, Nigeria. Plant extracts employed to control the vegetative mycelial growth were from neem (*Azadirachta indica*) and tobacco (*Nicotiana tabacum*) at 20%, 30%, 40%, 50% and 60% concentrations. In vitro application of extracts for the control showed that tobacco (*Nicotiana tabacum*) had fungitoxic effect that controlled the mycelia growth; there was complete inhibition ( $0.00 \pm 0.00$ ) at 60% on *Aspergillus viridae* and *Penicillium digitatum*. Tobacco extract on *Rhizopus* showed progressive retardations on the vegetative growth. Vegetative growth of the fungi on neem extract decreased with increase in concentrations; with its effect (extract) being felt more on *Penicillium digitatum* at 60% concentration ( $0.41 \pm 0.55$ ) when compare with the control. The inhibitory action of the two extracts on mycelial growth increased with increase in concentrations; giving toxicity profile of 60% > 50% in that order, and were significant at 0.05%.

**Keywords:** Tomato, fungi, plant extracts, vegetative growth, inhibition.

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### **INTRODUCTION**

The tomato *Lycopersicum esculentum* is an herbaceous, usually sprawling in the solanaceae or night shade family. It is a berry consisting of seeds with a fleshy pericarp developed from the ovary. The tomatoes, *Solanum lycopersicum syn Lycopersicum* formerly *Lycopersicon esculentum* is a plant in the family solanaceae of central south, and southern north America from Mexico to Peru [12]. Tomato was said to be a native of South America. Genetic evidence show that the progenitors of tomatoes were herbaceous green plants with small green fruit; with a centre of diversity in the highland of Peru [12]. One species, *Solanum lycopersicum* was transported to Mexico where it was grown and consumed by pre-historic humans. However, the fruit, grown by the Aztec of central Mexico, who called it 'xitomati' meaning plump thing with a nevel and later called tomato by other Meso-american people [11].

In Nigeria, tomato is a very popular vegetable crop. The fruit/berry can be used cooking, eaten fresh as salad; it can also be used as garnish. However, some very important destructive fungal diseases of tomatoes in the savanna area of Nigeria are *Alternaria alternata*, which is wide spread in tropical Africa. A very damaging pathogen in savanna areas is anthracnose disease induced by *Colletotrichum cingulata* [11]. Rhizopus rot induced by *Rhizopus stolonifer*, recognized in Nigeria (in the North), Upper Volta, Benin Kenya and in some Asian countries. Other diseases of importance to tomato are leaf spot induced by *Septoria lycopersici*; fruit rot, induced by *Rhizoctonia solani*. Among the field and storage tomato fruit fungi in Nigeria include *Colletotrichum coccodes*, *Botrytis cinerea*, *Xanthomonas campestris* *Pseudomonas syringae*, *Alternaria alternata*, *Phytophthora drechsleri* [4]. Other storage fungi affecting tomato include *Aspergillus* Sp, *Rhizopus* Sp and *Fusarium* Sp, and also produces aflatoxins which in some cases, very dangerous to human health [7].

Plant extracts have been found useful and less harmful to man and animals as well as the environments reports [6], has shown that compounds from plant sources are moderately toxic and are suitable as fungicides. Historical successes recorded in the use of Azadirachtin from neem plant (*Azadirachta indica*), nicotine from tobacco plant (*Nicotiana tabacum*) as bio-pesticides have spurred scientists to search for alkaloids, flavonoids, terpenoids and other secondary compounds, as bio-pesticides which are like to prove effective and environmentally safe. At least, 1,425 plant species have been found to possess bioactive compounds. Available literatures indicate that many of these plant species are effective against stored products pests [5]; [8]. Thus, this study is designed to isolate and identify fungi responsible for the fruit rot of tomato in five major towns in Kogi state, and to a large extent, systematically screen some locally available plant extracts, as natural products for their suitability and efficacies as bio – fungicides for the control of fruit rot of tomato as a single component control measure.

## MATERIALS AND METHODS

### Survey of fruit rot of tomatoes and samples collection

The survey was carried out between July and October 2009 in five major towns (Lokoja, Kabba, Okene, Anyigba and Ankpa) in Kogi State, where samples were collected and kept in a sterilized container in the laboratory for investigation

### Isolation and Identification of Pathogens

Diseased portion of the fruits were scrapped with the aid of scarpel, the segments were plated on petridishes containing PDA. The inoculated petridishes were incubated at room temperature and daily observations were made. The isolates were sub-cultured to get pure culture and then slanted in Mac-carthney bottles and kept for further experimental purposes. The fungi isolates were identified under the microscope, using lactophenol in cotton blue as the staining agent based on morphological characteristics and with reference to [2]; [3]. A pathogenicity test was carried out by inoculating healthy and fresh tomato fruits with mycelial suspensions according to the method of [1].

### Plant Extracts Employed.

Leaves of tobacco (*Nicotiana tabacum*) and neem (*Azadirachta indica*) were used. Fresh samples of each were used for the organic solvent (methanol) extractions following the methods of [9]. The homogenized powder of each was soaked in flasks containing methanol and thoroughly mixed together using glass rod and left for 24 hours to allow for extraction of the active ingredients as cold extraction; before filtering into a fresh 500ml flask using four – fold cheese cloth. The filtrates was concentrated using the vacuum evaporator so as to regenerate the

methanol. It was filtered using Buckner funnel and dried solidified extracts weighed. From the dry extracts, 13.34g, 20g, 26.67g and 33.33g were weighed separately and dissolved in 50ml distilled water to give the final concentrations of 20%, 30%, 40%, 50% and 60%. The bioassay of the plant extracts at these concentrations was carried out by determining the effects on radial growth inhibition.

## RESULTS

Three (3) different species of fungi isolated and identified to be associated with fruit rot of tomatoes are *Aspergillus viridae*, *Penicillium digitatum* and *Rhizopus* sp. They were found to be pathogenic using Koch's postulate. From the results, it was observed that the two aqueous leaf extracts used for the study recorded retardation or inhibition of mycelial growth of the fungi. The *in vitro* application of extracts showed tobacco to be more effective on vegetative growth of *Aspergillus viridae* and *Penicillium digitatum*. Complete inhibition of growth was recorded at 60% concentration (**Table 1**). Tobacco extract on *Rhizopus* showed progressive retardation on the vegetative growth. The effect of neem extract on the fungi however showed that the effectiveness in vegetative growth decreased with increased in concentrations (**table 1 and 2**).

**TABLE 1: The Inhibitory Effects of Tobacco Leaf Extracts on the mycelial growth of the fungi**

Concentration (%)	<i>Aspergillus viridae</i>	<i>Penicillium digitatum</i>	<i>Rhizopus sp</i>
20	1.20 ± 1.00	2.076 ± 1.66	2.16 ± 1.37
30	0.66 ± 0.60	1.80 ± 1.64	1.24 ± 1.22
40	0.42 ± 0.57	1.40 ± 1.59	1.18 ± 1.07
50	0.26 ± 0.34	0.21 ± 0.31	0.52 ± 0.78
60	0.00 ± 0.00	0.00 ± 0.00	0.48 ± 1.07

**Table 2: The Inhibitory Effects of neem leaf extracts on the mycelial growth of the fungi**

Concentration (%)	<i>Aspergillus viridae</i>	<i>Penicillium digitatum</i>	<i>Rhizopus sp</i>
20	2.78 ± 2.60	1.77 ± 1.63	1.55 ± 1.77
30	1.52 ± 1.86	0.78 ± 1.02	1.26 ± 1.60
40	1.12 ± 1.78	0.65 ± 0.58	1.16 ± 1.50
50	1.08 ± 1.73	0.51 ± 0.78	0.80 ± 1.45
60	0.74 ± 1.00	0.41 ± 0.55	0.78 ± 1.02

## DISCUSSION

According to the experimental work on the fungi associated with fruit rot of tomato, three species of fungi were isolated and identified. The result of pathogenicity test showed that all tomato fruits tested shows symptoms of rot, while all the uninoculated control fruits showed no symptoms of rot. The causal agents of the fruit rot was re-isolated and identified as *Aspergillus viridae*, *Penicillium digitatum* and *Rhizopus* sp thus fulfilling Koch's postulates.

The conidiophores of *Aspergillus* is upright, simple, terminating in an elevated swelling bearing phialids at the apex or radiating from the entire surfaces with conidia one-celled. *Penicillium* is typically consist of a highly branched network of multinucleated septate, usually branched conidiophores. Near the apex was a brush – like conidia – bearing apparatus, ending in phialids with I – celled, globose or ovoid conidia. Tobacco Plant (*Nicotiana tabacum*) was reported to be used for the control of plant diseases being ecologically friendly [10]. Study also showed that, tobacco as medicinal plant, posses potential antifungal properties which inhibit fungal mycelia

growth, hence the present result, where complete inhibition of mycelia growth ( $0.00 \pm 0.00$ ) was recorded at 60% concentration on *Aspergillus* and *Penicillium* cultures. Neem (*Azadirachta indica*) extract which is also economically friendly and medicinal can be used for the control of plant diseases. The present results showed that the mycelial growth of the three fungi decreased with increase concentrations.

### CONCLUSION

There is a dearth of information on the efficacies of most of the plant extracts against field crops pests and pathogens. Since nature has endowed the African continent with an array of plant showing geographical differences in the possession of biologically active compounds, the introduced plants in Nigeria's flora deserve further investigation.

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