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STUDIES ON THE FUNGI INVOLVED IN THE DETERIORATION OF STORED MELON SEEDS (*Citrullus colocynthis* (L.) SCHRAD IN ILORIN METROPOLIS AND CONTROL

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ABSTRACT

Studies were carried out to determine the organisms associated with the deterioration of melon seeds in storage in Ilorin, Nigeria. Five fungal species namely Aspergillus flavus, A. niger, Rhizophus stolonifer, Burgoa nigra and Fusarium sp. were isolated from deteriorating melon seeds. Studies on the antifungal activities of ginger and guava extracts on the isolates revealed that both radial growth and dry weight growth were hindered in all the isolates. Inhibition effects were dependent on the concentration of extracts. The results suggest that the test extracts could be handy in plant disease control and in planning the extention of the shelf life of melon seeds in storage.

INTRODUCTION

Colocynth (melon) also known as bitter apple, bitter cucumber, egusi, or vine of Solomon, is a vine plant native to the Mediterranean Basin and Asia especially Turkey, Nubia and Trieste. The original scientific name was *Colocynthis citrullus*, but is now classified as *Citrullus colocynthis* L. (Schrad.) (1). The colocynth plant is a creeping annual plant, native to arid soil and thrives well on rich light soil in the hot climate regions of Africa. In the south-eastern region of Nigeria, melon is best cultivated after the first rain of each year. The first fruits are harvested about thirteen weeks after planting (2).

The melon plant is monoecious and has large, fleshy, perennial roots, which send out slender, tough, angular, vine-like stems. The leaves are angular and lobed with yellow, solitary flowers in the axils of the leaves. The female flowers are readily distinguished by a globose, hairy, inferior ovary. The fruit is globular, smooth with a hard but thin rind, somehow like a gourd and filled with a soft white pulp in which are embedded numerous seeds. This pulp has medicinal value (3).

C. colocynthis, is a widely cultivated and consumed oil seed crop in West Africa (4). The plant is of the family Cucurbitaceae, a large family consisting of nearly 100 genera and 750 species (2 and 5). This plant family is known for its great genetic diversity and wide spread adaptation. Cucurbits are known for their high protein and oil contents. Seeds of cucurbits are sources of oils and protein with about 50% oil and up to 35% protein. The regions of cultivation are middle east, West Africa (Nigeria, Ghana, Togo, Benin) and other African countries for the food in the seeds and as crop inter-planted with maize, cassava and yam (2, 4 - 6).

Melon seeds, popularly called "egusi" in some parts of Nigeria, are edible and rich in fat and protein, eaten whole or used as an oil seed (1). Melon seeds are a good source of oil, protein, vitamins, minerals and energy in form of carbohydrates and contain 4.6g carbohydrate, 0.6g protein, 0.6g crude fiber, 33mg

vitamins, 17g calcium, 16mg phosphorus and 230mg potassium per 100g edible seeds (7). In Nigeria, melon seeds are consumed in various forms; as food ingredients or as soup thickener (egusi soup), melon ball snacks and ogiri (a fermented highly proteinous, soup condiment) (3, 4 and 7). In some rural parts of South-eastern Nigeria, the inhabitants mix milled melon with ground fungus *Pleurotus tuber-regium*, and mould them into stabilized balls to substitute meat in their diet.

Two types of melon seeds 'Bara' and 'Serewe' are commonly grown in southern Nigeria. They are distinguished on the basis of presence or absence of seed edges. 'Bara' is characterized by thick uniform seed edges with brown shells. 'Serewe' lacks pronounced seed edges but also with brown shells. The seed margin of 'Bara' is either black or white (6). Because of the high demand on melon seed in Nigeria, the tendency to store them for sale in time of scarcity is also high thereby making fungal infestation of stored seeds important.

One major challenge seeds face in storage is that of deterioration and several fungi have been implicated. Fungi of the genera *Aspergillus* and *Penicillium* are widely distributed storage fungi of egusi melon seeds, causing seed discolouration, decrease in nutritional value, increase in free fatty acid and peroxide values, decreased seed germination and producing a number of toxic metabolites, including aflatoxin (4). Basra *et al*. (8) reported that seed deterioration is manifested as a reduction in percentage germination while those seeds that germinate, produce weak seedlings.

In spite of all the technology available, the qualitative and quantitative losses originating during the post harvest process are still not well controlled and seeds are constantly exposed to external factors that encourage deterioration during storage. (9). Several methods may help upgrade the phytosanitary quality of seeds after harvest including surface disinfection, seed treatment, discarding of diseased seeds, hot water treatment and organic solvent infusion of antibiotics. The use of plant extracts in the

control of plant diseases is gaining importance because of the growing awareness on the hazardous effects of chemical fungicides to human health and environment (10 and 11). The aims of this study were to isolate and identify fungi associated with the deterioration of stored melon seeds in Ilorin and to determine the antifungal effects of guava leaves/ginger rhizome extracts on isolated fungi.

MATERIALS AND METHODS

Sample collection

Stored unshelled melon seeds were collected from two locations in Ilorin, Kwara State namely Oja-Oba and Ipata markets. Guava leaves and fresh ginger rhizomes used for the extracts were obtained from Sawmill and Tanke all in Ilorin.

Isolation of fungi from melon seeds

Melon seeds (the type with black edge) showing symptoms of infestation (discolouration, mold or distorted) from each market were carefully selected and labeled. Sixty seeds were randomly counted from the seeds from each location. Thirty seeds from each lot were shelled while the other thirty were unshelled. The different seed batches were surface-sterilized in 1% sodium hypochorite (NaOCl) for 1min and then rinsed in three changes of sterile distilled water (12 and 13). Identification of isolate(s) was based on the observed morphological features taking note of the growth rate and pattern on agar plates, colony size and colour and shape of spores, where produced (14 and 15). Stock cultures of the isolates were maintained in McCarthney bottle slants and stored for subsequent use.

Spore suspensions of the isolates were prepared (13).

Preparation of plant extracts

This was done according to the method of Malabadi and Kumar (16). The stock solutions were left for 24hr on laboratory bench

and later filtered aseptically into conical flasks. The extracts were stored in a refrigerator and used as required.

Effect of plant extracts on radial growth

The mycelial plug-radial growth technique was used (17).

Dry weight

Czapeks basal medium amended with different concentrations of the test extracts was used for this experiment (18).

RESULTS

Isolation

Five different fungi were isolated from deteriorated melon seeds jn this study namely *Aspergillus flavus, A. niger, Rhizophus stolonifer, Fusarium* sp. and *Burgoa nigra.*

Effect of plant extracts on radial growth

The inhibitory effects of aqueous, ethanolic and acetonic extracts of ginger and guava at different concentrations were evaluated on the following fungal isolates *A. flavus, A. niger, B. nigra, R. stolonifer* and *Fusarium* species. Aqueous guava extracts inhibited mycelial growth of *A. flavus* at concentrations 15% and 25% but not at 5% (Fig. 1). Aqueous, ethanolic and acetonic extracts of ginger and guava also inhibited the radial growth of *A. flavus* with the effect increasing with increase in the concentration of extracts. Aqueous guava extracts was observed to have the greatest inhibitory effect on the radial growth of the test fungi. This was followed by acetonic ginger extracts which inhibited the growth of *A. niger, A. flavus, R. stolonifer* and *Fusarium sp.* but had no significant effect in inhibiting the growth of *B. nigra.* Aqueous ginger extracts showed the least inhibitory effect on test fungi. Mycelial growth of *A. niger* was strongly hindered by 25% concentration of the different extracts (Fig. 2). Inhibitory effect of the test extracts on the growth of *B. nigra* was found to be minimal (Fig. 3). The reactions of the different isolates to the different

concentrations of test extracts varied according to concentration (Figs. 4 and 5).

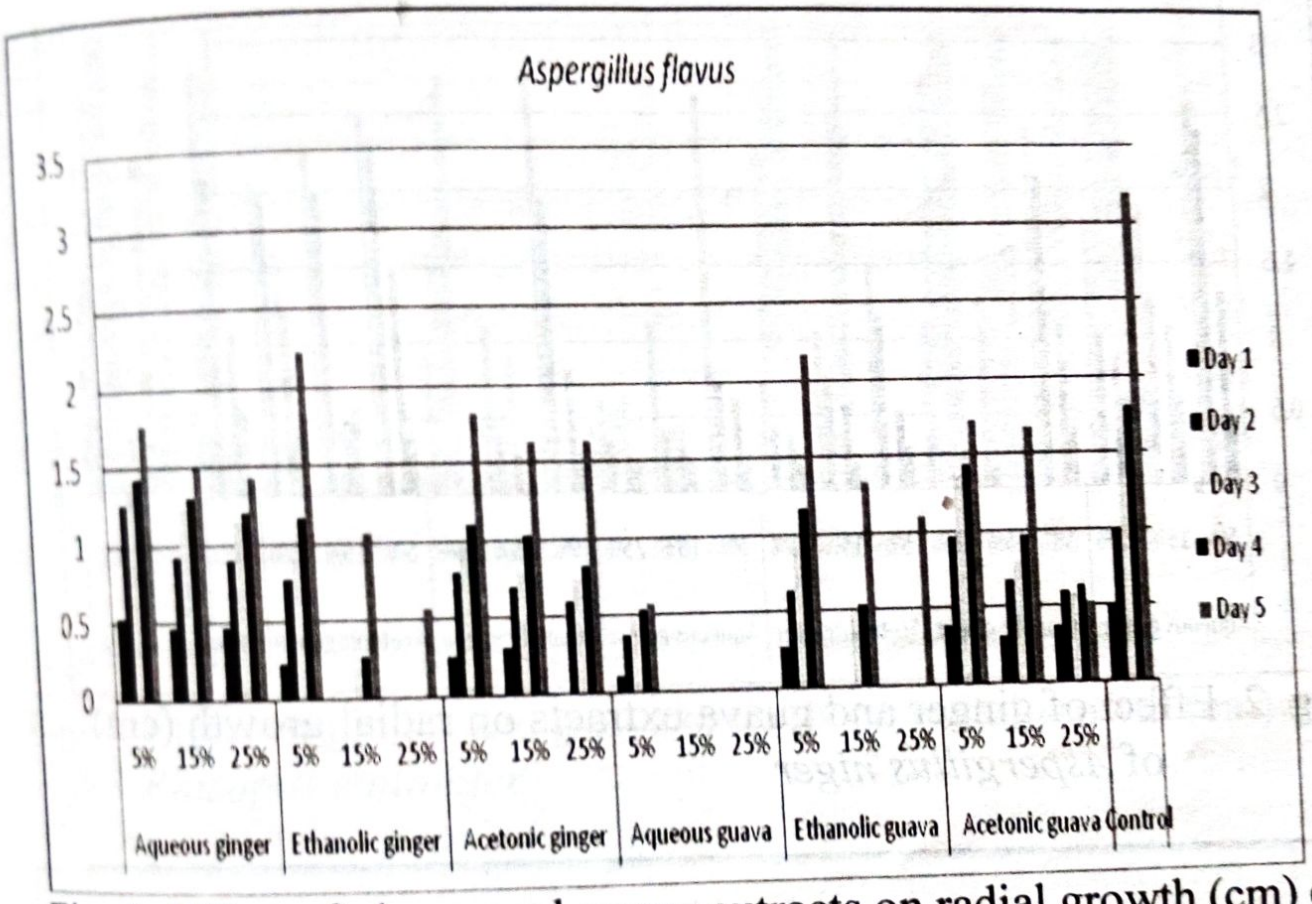

Fig. 1. Effect of ginger and guava extracts on radial growth (cm) of *Aspergillus flavus*

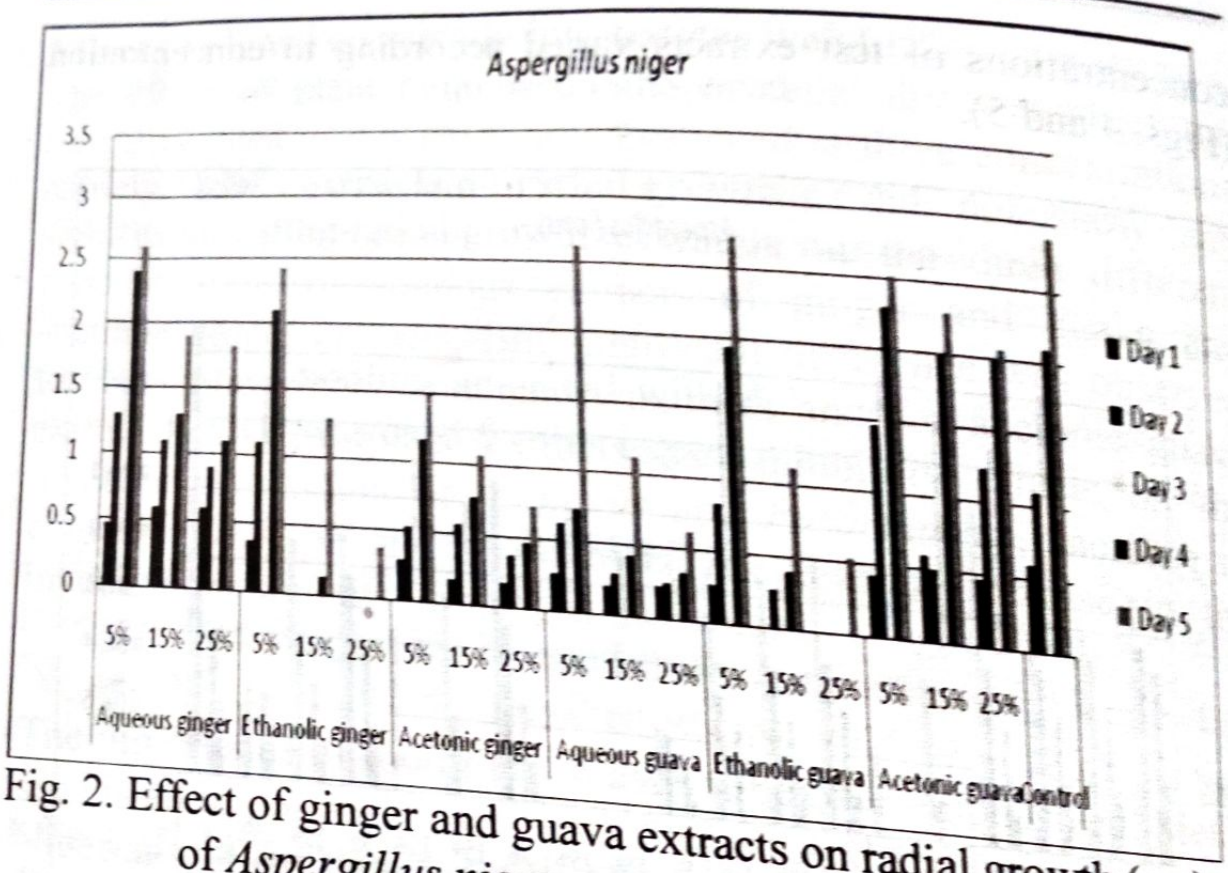

Fig. 2. Effect of ginger and guava extracts on radial growth (cm) of *Aspergillus niger*

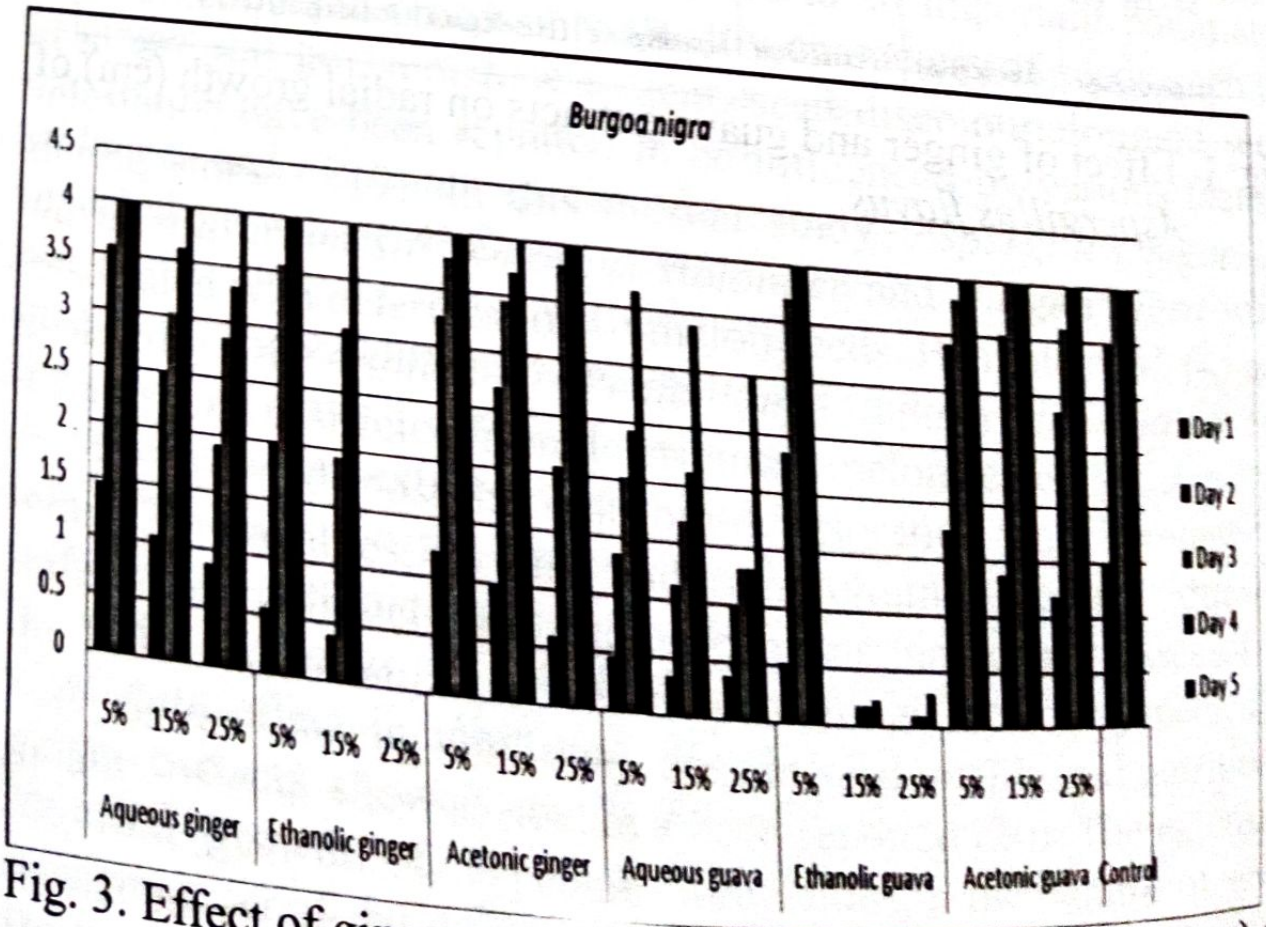

Fig. 3. Effect of ginger and guava extracts on radial growth (cm) of *Burgoa nigra*

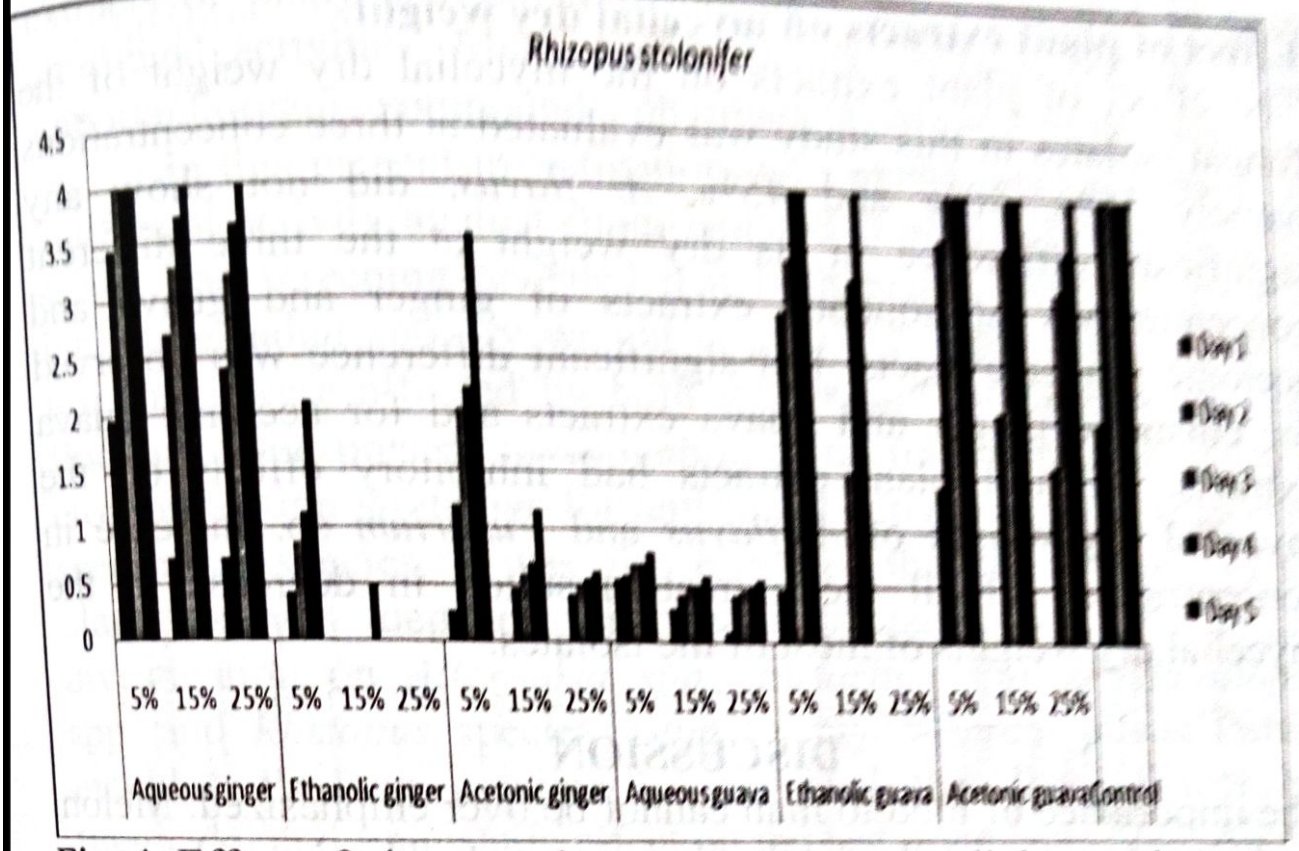

Fig. 4. Effect of ginger and guava extracts on radial growth (cm) of *Rhizopus stolonifer*

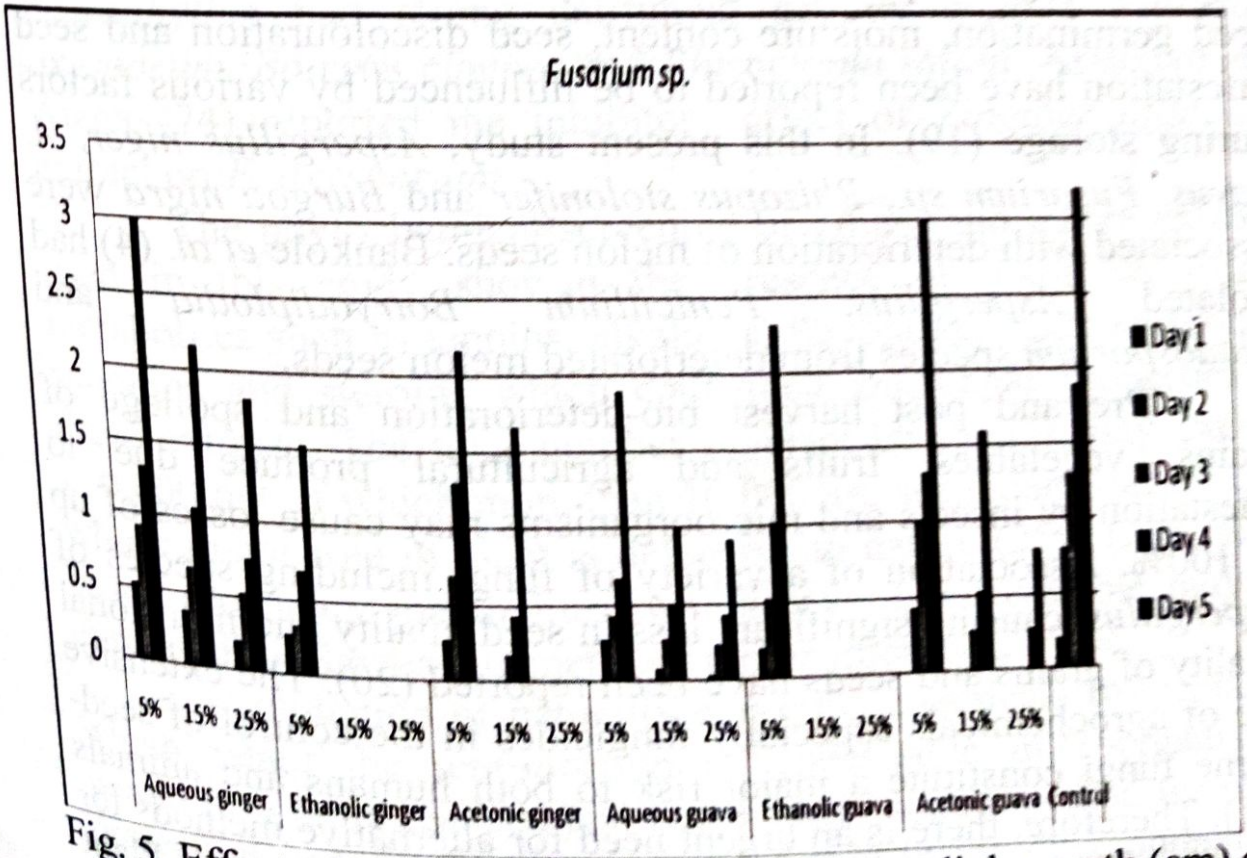

Fig. 5. Effect of ginger and guava extracts on radial growth (cm) of *Fusarium* sp.

Effect of plant extracts on mycelial dry weight.

The effect of plant extracts on the mycelial dry weight of the fungal isolates in this study was evaluated at three concentrations namely 15%, 30% and 45%. *A. flavus,* did not show any significant difference in its dry weight at the three different concentrations of aqueous extracts of ginger and guava and acetonic ginger extracts. But significant difference was observed for ethanolic ginger and guava extracts and for acetonic guava extracts. All test plant extracts had inhibitory effects on the mycelial dry weight of *A. flavus* and *Fusarium* sp. Increase in concentrations of all the extracts resulted in decrease in the mycelial dry weights of most of the isolates.

DISCUSSION

The importance of food to man cannot be over emphasized. Melon seed is an important oil seed that is consumed and enjoyed by most Nigerians. The quality of melon seed is an important parameter for marketing and processing. Quality characters of seeds such as seed germination, moisture content, seed discolouration and seed infestation have been reported to be influenced by various factors during storage (19). In this present study, *Aspergillus niger, A. flavus, Fusarium sp., Rhizopus stolonifer* and *Burgoa nigra* were associated with deterioration of melon seeds. Bankole *et al.* (4) had isolated *Aspergillus, Penicillium Botryodiplodia* and *Cladosporium* species from deteriorated melon seeds.

Pre and post harvest bio-deterioration and spoilage of grains, vegetables, fruits and agricultural produce due to infestation by insects and microorganisms may cause losses of up to 100%. Association of a variety of fungi including species of *Aspergillus,* causing significant loss in seed quality and nutritional quality of grains and seeds have been reported (20). The extensive use of agrochemicals especially fungicides in the control of seed-borne fungi constitute a major risk to both humans and animals (21). Therefore, there is an urgent need for alternative methods for handling the problems of deterioration of seeds in storage. Plant

extracts of many species of plants have been reported to have antifungal activities. Many higher plants produce economically important organic compounds, pharmaceuticals and pesticides.

In this present investigation, two plants were screened for antifungal activity against fungi isolated from deteriorated melon seeds. The screening revealed that both plants were effective in inhibiting radial and dry weight growth. Mycelial dry weights of test fungi were affected by both ginger and guava extracts. The finding of the present investigation is an important step towards crop protection strategies for antifungal activity against important seed-borne species. It was also observed that the efficacy of the plant extracts depends on the extractant used. A similar investigation on *Alternaria spp.*, *Fusarium spp.*, *Pestalotiopsis* spp. and *Rhizopus* species using twenty different plants (which included *Psidium guajava* L.) revealed that these plants had fungicidal and fungistatic effect on the test organisms (22). Alkhail (23) showed that aqueous extracts of *Allium sativum, Cymbopogon proxims, Carum carvi, Azadirachta indica* and *Eugenia caryophyllus* had strong antifungal activity against *Fusarium oxysporum, Botrytis cinerea* and *Rhizoctonia solani.* Amienyo and Ataga (24) reported the inhibitory effect of *Zingiber officinale* extract on *F. oxysporum.*

The phytochemical screening of ginger and guava extracts used in this study showed the presence of some secondary metabolites such as tannins, alkaloids, flavonoids, anthraquinones, saponins and steroids which confirmed antifungal properties in these extracts. The total number of plant chemicals may exceed 400,000 out of which more than 10,000 are secondary metabolites whose major role in plant is defensive in nature (25). Thus, plant based secondary metabolites, which have defensive role may be exploited for the management of storage microbes.

Exploitation of naturally occurring chemicals from plants, which retard the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of

future commercial pesticides for crop protection strategies (20). This study has shown that the use of extracts from ginger rhizome and guava leaves to control fungi associated with deterioration of stored melon seeds has potential as a substitute for chemical fungicide. This approach to plant disease management is economically viable and poses little environmental risk and the plants are available to farmers in Nigeria that do not have ready access to other synthetic fungicides.

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