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Effect of Benlate Solution, Crude Leaf Extracts of *Azadirachta indica* and *Ocimum gratissimum* on Growth of Fungi and Preservation of Melon Seeds

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Abstract: The effect of crude leaf extract of *Azadirachta indica*, *Ocimum gratissimum* and benlate solution on growth of pathogenic fungi isolated from *Cucumeropsis mannii* (Melon) seed was studied. Appropriate means of applying the leaf extract to preserve the melon seed was also investigated. The leaf extracts and benlate solution reduced the growth of the fungi with increase in concentration and were effective in preserving melon seeds against fungi for six months. *Ocimum gratissimum* leaf extract reduced fungal growth more than that of *Azadirachta indica*. The benlate solution reduced the fungal growth rate most, completely stopping growth at 1.0 and 0.7% concentrations. Melon seeds treated with powdered leaves had no infection when infected with fungi as against seeds treated with ethanol leaf extracts of the medicinal plants which had 16.4% infection when inoculated with *Absidia blakelseeana*, while the control had 100% infection for each of the fungus. There was a significant reduction of germination when seeds were treated with ethanol leaf extracts. However, seeds treated with powdered leaves of the plants favoured 100% germinations.

Key words: Preservation, crude extracts, fungi, melon seed, germination

INTRODUCTION

Cucumeropsis mannii Naud-Holl ('Egusi' Melon), in the family cucurbitaceae, seeds and its oil serve as a rich source of dietary energy. The melon soup is a favourite of many people in West Africa[1]. Melon seed oil may be used as a base for soap and body cream production[2]. Seed deterioration by fungi, bacteria, insects, virus and other plant pathogens occurs during storage[3], fungi being the commonest agents[4]. Adekunle[5] and Adekunle and Uma[6] reported the isolation of some pathogenic fungi and their biochemical effect on melon seeds.

Chemical control of seed borne pathogens with fungicides is an effective method. However the disadvantage is that these chemicals are toxic to mammalians, man inclusive[7]. Benlate (Methyl-I-Butyl-carbomoyl 1-2-benzimidiazole carbamate) is a relatively safe, most important broad spectrum systemic fungicide against a large number of important fungal pathogens and it also suppresses mites. The recommended dosage of benlate is 5 parts per thousand (0.5% concentration). At this concentration it is toxic to fungi but not toxic to mammals and less phytotoxic[8]. Local medicinal plants used to cure other ailments, which possess antifungal substances and not toxic to mammals at any concentration, would be a better means of suppressing fungal growth than orthodox chemicals such as benlate[9], hence the need to study preservation of seed and grain using medicinal plants.

Azadirachta indica L. (Neem tree) and *Ocimum gratissimum* Willd-Holl ('Effirin') are some of the medicinal plants used in Nigeria. The neem leaf are used to rub the body for protection against fungal attack, some people even use the leaf extract for bathing to protect the skin against fungal diseases generally[10]. The active components isolated from Neem include Triterpenoids, Azadirachtin and Melantriol[11], all of which suppress feeding of pests. Thymol has been identified as the active ingredient of *Ocimum gratissimum* and has been found to suppress fungal growth[9].

Makanjuola[12] used methyl spirit and water crude extract of neem leaves and kernels to protect cowpea and maize against two insects for 5 months. The experiments showed that the extracts suppressed the growth of insects and helped in preserving the seeds. Although crude extracts from Nigerian medicinal plants including *Azadirachta indica* and *Ocimum gratissimum* have been used to suppress fungal growth[9] their effect on storage of seeds and grains have not been reported. The objective of this study therefore, were to determine the effect of crude leaf extracts of *Azadirachta indica* and *Ocimum gratissimum* and benlate solution on growth of

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fungi isolated from diseased melon seeds. Also it was aimed at determining the appropriate means of applying the leaf extract of the plants to preserve melon seeds in storage.

MATERIALS AND METHODS

Effect of crude leaf extracts of *Azadirachta indica*, *Ocimum gratissimum* and benlate solution on growth of fungi isolated from melon seeds: Fresh leaf extracts and dry leaf extract of the herbs were used for this study. A preliminary study was done to select the best solvent from absolute ethanol, acetone, chloroform, methyl spirit and water, respectively. The absolute ethanol extract was found to be most effective so it was used for further study.

Fresh leaf extract: Modified method of Butterworth and Morgan[11] was used here. The fresh leaves of each was blended in an electric blender (Model: T 1348). To 20 g of this was added 20 ml of solvent (Ethanol), they were then shaken in an electric shaker for 30 min. After that the solvent supernatant was decented into an evaporating dish and placed in a water bath to dry. To the residue was added 20 ml of sterilized distilled water. This served as the crude extract of fresh leaf for each plant.

Dry leaf extract: A modified method of Makanjuola[13] was used. Leaves of *Azadirachta indica* and *Ocimum gratissimum* were collected and air dried separately in the laboratory for 14 days. Each was then blended into powder. Ten grams of the powder was soaked in 100 ml of absolute ethanol (plant solvent ratio 1:10) for 24 h. The solution was then filtered into a beaker with the aid of a sterilized muslin cloth. The filterate was concentrated in a rotatory evaporator. This was used as crude extract. The extract was covered with aluminium foil and kept in the fridge until needed. Just before use, serial dilutions for each medicinal plant were prepared for the following concentrations: 70, 50, 30 and 10% (using 70% ethanol solution).

The effect of crude extracts of *Azadirachta indica* and *Ocimum gratissimum* on fungal growth was done by infusing 1 ml portion, with a pipette, of the extract (100%) and the serial dilutions (70, 50, 30 and 10%) of this extract into petri dishes (9 cm in diameter) containing 8 ml of potato dextrose agar (PDA) previously prepared. To another set of petri dishes, benlate solutions of 1% (1 g benlate in 100 ml distilled water); 0.7% (0.7 g benlate in 100 ml distilled water); 0.5, 0.3 and 0.1% concentrations were added, respectively. The leaf extracts and benlate solutions solidified with the PDA. The inoculation of the fungi was done by using sterile cork borer (0.5 cm in diameter). The cork borer was used to cut 2 weeks old fungal plate into the fresh PDA plate. A control experiment in which water was added in place of crude extracts or benlate solution solidified with PDA. The inoculation of the fungi was done by using sterile cork borer (0.5 cm in diameter). The cork borer was used to cut 2 weeks old fungal plate into the fresh PDA plate. A control experiment in which water was added in place of crude extract or benlate solution was set up. Five replicates were used for each fungus and control, respectively. The petri dishes were incubated for 8 days at room temperature (28-30°C). The daily radial growth was taken and recorded during the period of incubation according to Booths[14].

Effect of *Azadirachta indica*, *Ocimum gratissimum* crude extracts and benlate solution on deterioration of melon seeds: To test for the effect of the extracts and benlate solution on the infection of melon seeds, seven thousand visually healthy melon seeds were soaked for 6 h in each of the crude leaf extracts (1000 seeds in 50 ml of the ethanol leaf extract). Another seven thousand seeds was soaked in benlate solution (0.5%), respectively. They were air dried in the laboratory for 48 h. After this, a set of 1,000 seeds of each treatment were inoculated with 2 weeks old culture on each of the following fungi: *Absidia blakelseeana*, *Aspergillus flavus*, *Fusarium solani*, *Macrophomina phaseolinea*, *Pencillium chrysogenum* and *Rhizopus oryzae*, previously isolated by the authors from diseased melon seeds. This was done by placing the seeds on the fungal plate for 24 h. Two batches of control was done, the first was made using 6 sets of 1,000 seeds soaked in each crude extract concentration separately and treated as reported above without inoculating with a fungus on the PDA plates. The second control was set up with 7 sets of 1,000 seeds that were not treated with either the crude extracts or benlate solutions. A set of 1,000 seeds was placed on each of the six fungal cultures and the PDA plates without any fungus and incubated for 24 h. After the 24 h incubation, all these sets of seeds were put in separate polythene bag, marked accordingly and stored for 6 months. Another set of 12,000 seeds were inoculated with the six fungal isolates (2,000 per isolate). A set of 1,000 seeds were placed in 50 g of ground dried leaves of each medicinal plant used in separate polythene bags and stored in the laboratory for six months. For the control of this experiment, a set of 1,000 uninfected seeds were placed in a polythene bag containing 50 g of the dried leaves of medicinal plant. They were also stored for six months.

After six months of storage, a sample of 1,000 seeds from each batch of seed was plated out on previously

prepared semi-liquid agar plates (10 seeds per plate) and labeled accordingly. The plates were incubated at room temperature. After 8 days, the number of infected seeds were recorded and percentage infection calculated for each treatment. To test the effect of the leaf extracts, ground leaves and benlate solutions on the germination of melon seeds after the storage period, 400 treated seeds of each crude extract and benlate solution was sampled. The method of Agrawal[15] was used for the germination studies. The results were statistically analysed for comparison as described by Parker[16].

RESULTS

All the fungi responded to concentration change of the leaf extracts and benlate solutions. The extracts and benlate solution reduced or completely stopped the fungal growth as compared to the control (Fig. 1-3). There was a greater growth of fungi at low concentrations (0.1 or 10%) benlate leaf extract concentrations, respectively) that at high concentrations (1 or 100%). The *Ocimum gratissimum* leaf extract reduced fungal growth rate more than the *Azadirachta indica* leaves (Fig. 1 and 2). Some fungi at 100% *Ocimum* leaf extract concentration did not grow at all, they were *Absidia blakelseeana*, *Macrophomina phaseolina* and *Fusarium solani*. However they grew for other *Ocimum gratissimum* leaf extract concentrations. Benlate solution reduced the fungal growth rate most, completely stopping growth at 1.0 and 0.7% concentrations (Fig. 3). At 0.5% of benlate concentration 2 fungi grew, *Absidia blakelseeana* and *Macrophomina phaseolina*. Subsequently there were growth for all fungi in the 0.3 and 0.1% benlate solutions. The daily growth rates of the fungi shows that these leaf extracts and benlate solutions at 100 and 0.5% caused delay in growth of fungi for at least 2 days. Thereafter they resumed normal growth, although there was a reduction in the growth rate compared with the control. There was an increase in fungal growth rate as the benlate and leaf extract concentration decreased from 1.0 or 100% to 0.1 or 10%, respectively (Fig. 1-3).

After 6 months incubation all the melon seeds treated with leaf extracts (Ethanol or ground dried leaf) and benlate solutions showed on infection except *Macrophomina phaseolina*, which had 5.0% infection with benlate treatment (Table 1) and also *Absidia blakelseeana* caused 16.4% infection on seeds treated with alcohol extract of *Azadirachta indica*. *Ocimum gratissimum* (83% germination) had significant reduction in germination, with *Azadirachta* extract causing the greatest decrease in germination, 23% to the control. The leaf ground dried leaf of the two plants favoured 100% germination of melon seeds.

DISCUSSION

These investigations have shown that leaf extracts from *Ocimum gratissimum* was more efficient in reducing fungal growth than that of *Azadirachta indica*. This was revealed when three of the fungal isolates did not grow at 100% concentration of *Ocimum* leaf extract (Fig. 1) while all the fungal at the same concentration of *Azadirachta* leaf extract grew (Fig. 2). With increase in concentration the plant extracts becomes more efficient in controlling fungal attack on melon seeds, the growth rate of the fungi were reduced. The 100% concentration being the most effective.

This study confirms that *Ocimum gratissimum* and *Azadirachta indica* leaf extracts have fungistatic properties as suggested by Areo[9]. The fact that the leaf extracts were able to control a wide range of fungi might suggest that they posses broad spectrum fungistatic properties. The efficacy of their fungistatic properties is as good as that of the check fungicide, benlate. The slight differences which occurred during the fungal growth experiment might be due to the fact that it was the

Table 1: Effect of leaf extracts of *Azadirchta indica* and *Ocimum gratissimum* as well as benlate solution on deterioration of stored melon seeds

	Percentage infection of melon seeds after six months storage (%)					
	No. of treatments	*Azadirachta indica* (seeds soaked in liquid extract)	*Azadirachta indica* (leaf powder)	*Ocimum gratissimum* (seeds soaked in liquid extract)	*Ocimum gratisssimum* (leaf powder)	Benlate (0.5%, (w/v) 0.5 g in 100 ml of distilled water
Control	0.00	0.00	0.00	0.00	0.00	0.00
Absidia blakelseeana	100.00	16.40	0.00	0.00	0.00	0.00
Aspergillus flavus	100.00	0.00	0.00	0.00	0.00	0.00
Fusarium solani	100.00	0.00	0.00	0.00	0.00	0.00
Macrophomina phaseolina	100.00	0.00	0.00	0.00	0.00	0.00
Pencillium chrysogenum	100.00	0.00	0.00	0.00	0.00	0.00
Rhizopus oryzae	100.00	0.00	0.00	0.00	0.00	0.00

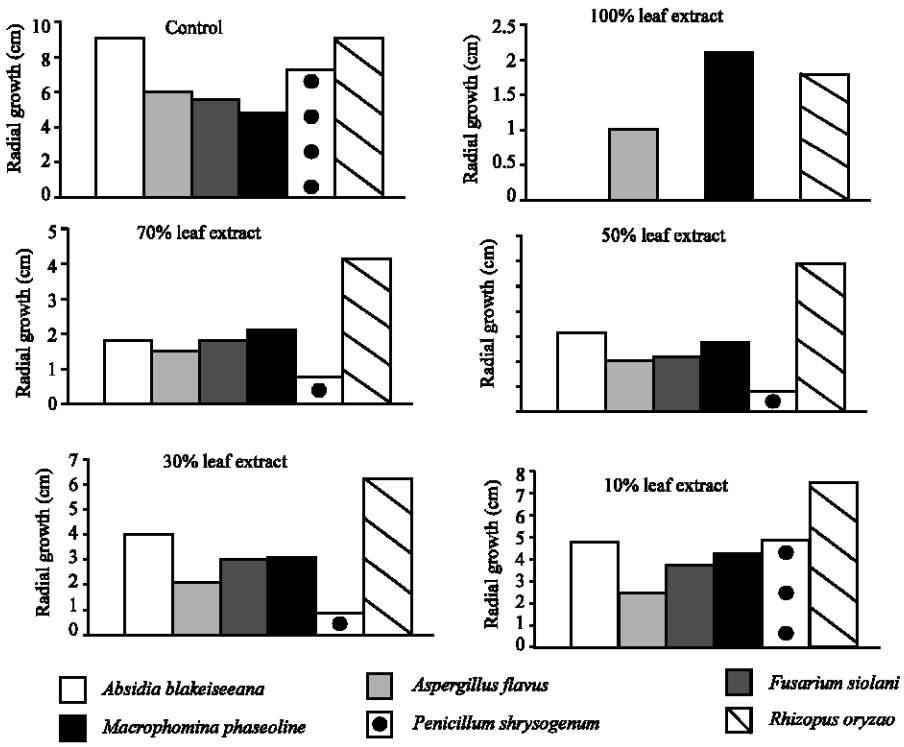

Fig. 1: Average radial growth of some fungi isolated from meion seeds on PDA treated with *Ocimum gratissimum* leaf extract at different concentration, after 8 days incubation

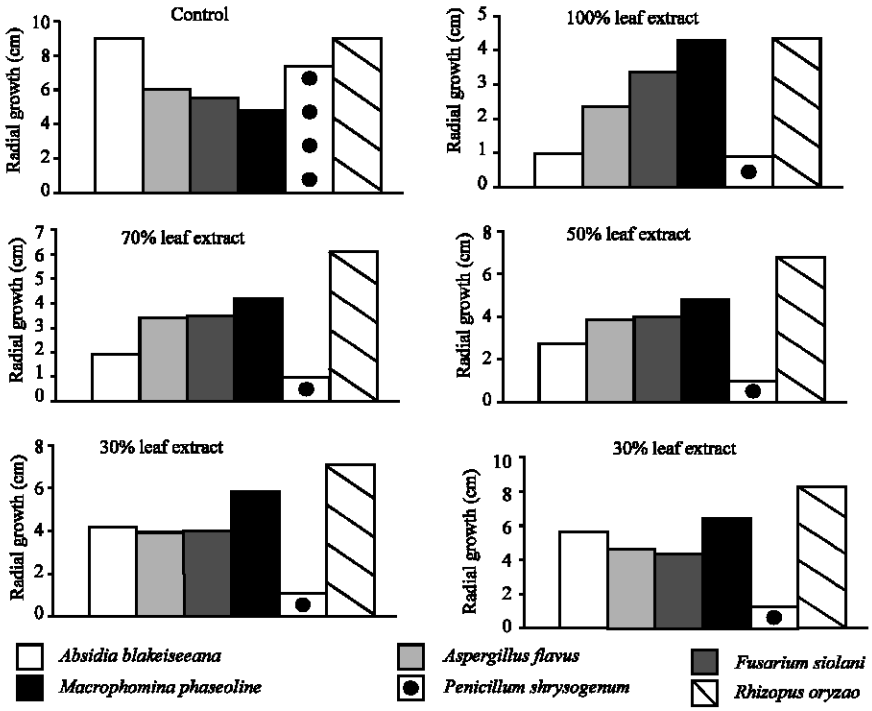

Fig. 2: Average radial growth of some fungi isolated from meion seeds on PDA treated with *Azadirachta indica* leaf extract at different concentration, after 8 days incubation

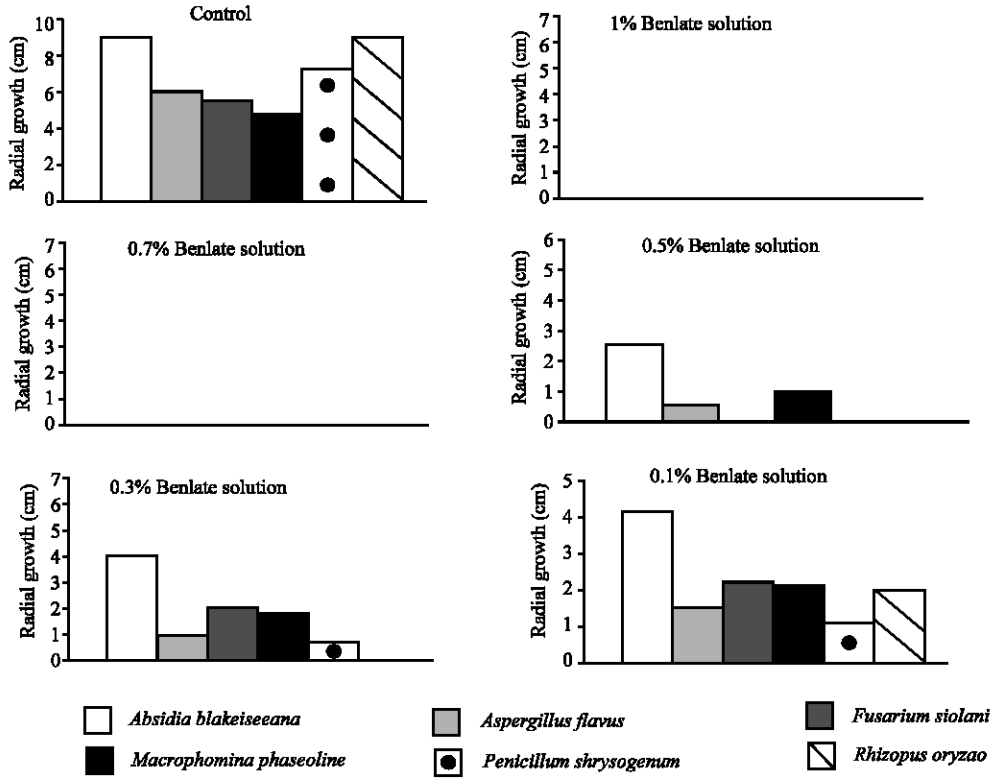

Fig. 3: Average radial growth of some fungi isolated from meion seeds on PDA treated with Beniate solution leaf extract at different concentration, after 8 days incubation

crude extract of the plants that was used and not the purified active ingredient. With the active ingredient it might be more efficient than the benlate. Even then the fact that the medicinal plants are not known to be toxic to man at all concentrations and are fungistatic, while the check fungicide benlate is toxic to man[17] makes the plants more suitable in controlling fungal attack on melon seeds during storage of particular interest is the fact that the ground dried leaves of *Ocimum gratissimum* and *Azadirachta indica* did not affect the germination of melon seeds after six months storage, implying that the medicinal plants administered in the powdered form did not affect this economic importance of storing melon seeds. However when the medicinal plants were administered in liquid form (soaking of seeds in liquid ethanol leaf extracts) they affected germination. This might be due to the fact that the alcohol might have caused dehydration of the embryo of the seeds as suggested by Neegaar[7].

From these observations storing melon seeds in powdered dried leaf of *Ocimum gratissimum* and *Azadirachta indica* against fungal attack can be effective and thus exploited.

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