In Vitro Evaluation Of Selected Plant Extracts As Biocontrol Agents Against Black Mold (Aspergillus Niger Van Tieghem) Of Onion Bulbs (Allium Cepa L.)

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Abstract: Black mold disease caused by Aspergillus niger V. Tiegh. is the most devastating infection occurs in onions (Allium cepa L.) under field and store conditions. The use of biocontrol agents is ecofriendly approach for controlling seedborne and soilborne diseases compared to the use of toxic synthetic fungicides. This study has been designed to assess the contamination levels of onion seeds with A. niger and its effect on seed germination, and to evaluate the in vitro antifungal activity of Prunus mahaleb seeds, Commiphora myrrha resin (0.5, 1.0, 1.5, 2.0 g/100 ml), Syzygium aromaticum dry buds (clove), and Panax ginseng roots extracts (0.5, 1.0, 2.0, 2.5 g/100 ml) against black mold of onion bulbs. The fungus is seedborne pathogen which significantly contaminated onion seeds (89-100%) and reduced seed germination (39-83%). The extracts of clove caused 43-96% inhibition in spore germination followed by mahaleb (37-96%), myrrha (33-88%), and ginseng (34-87%). The highest concentration of these extracts (3.0%) did not affect seed germination, but significantly reduced seed contamination by A. niger up to 84%, 80%, 71%, and 65% for Syzygium aromaticum, Syzygium aromaticum, Panax ginseng and Prunus mahaleb, respectively. The extracts apparently inhibited the fungal growth and mold development on stored onion bulbs which indicates the antifungal property of these extracts against A. niger. Therefore, they can be recommended as effective biocontrol agents to reduce seed contamination and enhance the storability of onion bulbs. Thus, the use of healthy and certified seeds for onion production is a priority.

Keywords: Allium cepa, Aspergillus niger, biocontrol, Commiphora myrrha, Panax ginseng, Prunus mahaleb, Syzygium aromaticum.

INTRODUCTION

Onion (Allium cepa L.) is an economically important vegetable crop cultivated annually for its leaves and bulbs which consumed as salad and cooked vegetable under both temperate and tropical regions [1-3]. The onion seeds and sets used for bulbs production are vulnerable to various fungal infections. These fungi affect seed germination, emergence from soil, seedling growth vigor and bulb storability [4-6]. In the Sudan, the onion production is evidently affected by the invasion of various pathogenic and saprophytic fungi leading to considerable losses during field growth and storage [1]. Black mold (Aspergillus niger Van Tieghem) is the main storage disease which contaminates more than 80% of the stored bulbs [7,8]. It is a limiting factor in onion production worldwide [9]. The main sources of black mold are seedborne, soilborne and airborne inocula which heavily invaded dead onion tissues and aggravated under inductive storage conditions [10-12]. The fungus transmitted from soil and naturally contaminated seed to onion seedlings and sets and eventually to bulbs [9,10]. The spore loads in the seeds and soil are correlated with the mold development on the bulbs [3,13]. Different agronomical methods were used for controlling the fungal diseases of onion plants.

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The use of synthetic chemical fungicides is a common practice around the globe. However, their toxicity and non-biodegradable nature limit their usage [14]. The seed treatments with fungicides were less effective in crop production in field regularly used for onion production [15]. Therefore, the use of biocontrol agents such as natural plant extracts and microorganisms is promising for controlling seed and soilborne diseases [3,16]. Several biocides (Bioagents and plant extracts) have been reported to be eco-friendly control measures comparable to synthetic fungicides. They increase seed germination and vigor index by reducing the pre- and post-emergence mortality in many crops including onion [9]. Seed treatment reduced the incidence of storage black mold in crops grown on land not previously used for onion production, but not sufficient to provide complete control [11,15]. Worldwide, many researchers testing the effect of various plant extracts as biocontrol agents for different plant diseases [17,18]. Antifungal activity of garlic (Allium sativum L.), onion (Allium cepa L.), and leek (Allium porrum L.) against A. niger was reported [16]. Many studies revealed the antimicrobial properties of different plant species with increasing attention on herbal, medicinal, and aromatic plants for their antifungal activities [19]. The Asian ginseng (Panax ginseng) and American ginseng (P. quinquefolium L.) had weak activity against Gram-negative and Gram-positive organisms as well as antifungal property [20]. P. ginseng releases antimicrobial polyacetylene into the surrounding soil from the root as defense compound against diseases [21]. Clove (Syzygium aromaticum) ethanolic extract exhibited high antimicrobial property against different bacteria and fungi [22-25]. The clove essential oil revealed moderate antifungal activity towards various pathogenic fungi [26-29]. Prunus mahaleb extracts had inhibitory effect at various concentrations against Gram-negative and Gram-positive bacteria [30], and demonstrate antifungal nature for many pathogenic fungi [31] Commiphora myrrha essential oil and gum resin extracts displayed antifungal and antibacterial

activities [32-38]. Realizing the antimicrobial property of these plant extracts, the present studies was therefore designed to investigate the contamination levels of onion seeds with A. niger and its effects on seed germination, and evaluating the potential role of four plant extracts namely Prunus mahaleb, Commiphora myrrha, Syzygium aromaticum, and Panax ginseng on spore germination, seed contamination/germination, and storability of onion bulbs.

Materials and methods

Collection of the seed samples

The seed samples of 14 onion (Allium cepa L.) cultivars were collected from different sources and locations in Sudan such as Sennar National Seed Association, Hudeiba Research Station, Gezira Research Station, Shawgi Seed Stores, Nile Seed Company, Islamic Agricultural Development Company, Arab Agricultural Foundation, Shendi city local markets, and from Sungi Island in the Nile province. The sample were derived from the three types of onion widely used in Sudan viz. “Saggai” which is red, “Nassi” (El-Hilo) white, and “Kamleen” red. The seeds samples were drawn and examined as adopted by the rules of the International Seed Testing Association (ISTA, 1966)[39].

Seed germination and contamination by A. niger

In the present study, the seed samples were tested for seed germination and contamination by A. niger using blotter and agar plate methods [39]. Four hundred seeds from each sample were surface sterilized by immersing them in a 1% solution of sodium hypochlorite for 5 minutes and followed by subsequent rinsing in several changes of sterile distilled water. The seed were then aseptically inoculated on Petri plates containing moist filter papers (Blotter), and potato dextrose agar (PDA). The inoculated plates were incubated in Gallenkamp illuminated incubator at 28 ± 2ºC with alternating cycle of 12 hours near ultraviolet light and darkness to stimulate the growth of the fungal flora. The blotters were kept moistened by adding sterile distilled water throughout the incubation period of 15 days. The percentages of seed germination and contamination by A. niger were reported.

Identification of A. niger

The predominant colonies of A. niger developed on onion seeds were identified using macroscopic characteristics on the growth media and the microscopic examination of the vegetative and reproductive structures as suggested by many authors [40].

Effect of plant extracts on the growth of A. niger spores

For evaluating the effect of plant extracts on the spore germination of A. niger, four plant species parts (seeds, resin, flower buds, and roots) were selected namely, Prunus mahaleb seeds, Commiphora myrrha resin, Syzygium aromaticum dry flower buds, and Panax ginseng roots. Different concentrations from each plant extracts were prepared using sterile distilled water. The desired weight from each plant part was soaked for 3 hour in water and then filtered through filter paper No. 1. Concentrations of 0.5, 1.0, 1.5, and 2.0% (w/v) were prepared from Prunus mahaleb and Commiphora myrrha, where 0.5, 1.0, 2.0, and 2.5% (w/v) were prepared from Syzygium aromaticum and Panax ginseng. From 10-day old culture of A. niger grown on PDA, 5 loop full inoculum were added to 9 ml sterile distilled water, mixed thoroughly and a serial dilutions were prepared up to 10^4. To each concentration of the plant extracts, 1 ml of the spore suspension was added in a test tube, mixed, kept for 5 minutes, and were then added to sterile plates and mixed with 10 ml of molten PDA. As a control, similar treatment was prepared by replacing the plant extract with sterile distilled water. Four replicates were incubated at ambient temperature (26ºC) for 7 days and the percentages of spore germination were calculated.

Effect of plant extracts on seed germination and contamination by A. niger

For testing the effect of four plants extracts (Prunus mahaleb, Commiphora myrrha, Syzygium aromaticum, and Panax ginseng) on onion seed germination and contamination by A. niger, concentration of 3.0% from each extract was prepared. Four hundred onion seeds were soaked in the extract for 5 min, dipped in several changes of sterile distilled water and inoculated aseptically on moistened filter papers (Blotter), and PDA. As a control, similar set of seeds was left without treatment. The inoculated plates were incubated at room temperature (26ºC) for a week and the percentages of seed germination and contamination were recorded.

Effect of plant extracts on mold development on stored bulbs

In order to assess the effect of the selected plant extracts (Prunus mahaleb, Commiphora myrrha, Syzygium aromaticum, and Panax ginseng) on A. niger growth and development on onion bulbs, concentration of 3% from each plant was prepared. Four lots of 10 bulbs each from red cultivar were dipped for 10 minutes in the plant extracts, and air dry. As a control, another set of bulbs was left without surface sterilization. The bulbs were artificially inoculated by soiling with dry spores of A. niger [8]. Three replicates were kept in paper bags at ambient temperature (32ºC) for one month and the mold development on onion bulbs was observed.

Statistical analysis

A one way ANOVA test (correlation coefficient) was used to determine the variation between the effect of different concentrations of plant extract on A. niger spore germination, seed germination and contamination. The analysis was carried out using statistical package software SPSS (version 11.0).

RESULTS AND DISCUSSION

Black mold disease caused by A. niger V. Tiegh. is the main infection of onion seeds, seedlings and stored bulbs. The fungal spores from naturally contaminated onion seeds and soilborne transmitted to seedlings and caused 30-80% losses in onion bulbs [9-10]. The fungus significantly reduces seed germination, seedling emergence, and roots and shoots elongation causing pre- and post-emergence mortality [41]. The fungus filtrate reduced seed germination to 65.3% compared to control [42]. Seedlings from contaminated onion seeds had longer roots but shorter shoots [10]. The culture filtrate of A. niger exhibited phytotoxicity against onion, tomato, maize and groundnuts by reducing seed germination and root elongation [42-44]. In the present study, the seeds of the 14 cultivars of onion were highly contaminated by A. niger (89-100%), which apparently reduced seed germination (39-83%)

(Table 1) as concluded in similar mycological studies on onion seeds and other plants [10,41,42]. Therefore, the use of healthy and certified seed was recommended by many authors to reduce the amount of the spore inoculum. The use of synthetic chemical fungicides is a common practice extensively adapted to combat various plant diseases comparable to one extracted from different plant parts. Nonetheless, the goal of the recent research is focusing on alternative control strategies to the use of synthetic fungicides [14]. Several studies demonstrate the effectiveness of different plant extracts as excellent source of eco-friendly natural fungicides [45-49]. Plant extracts from Abrus precatorius, Aegle marmelo, Areca catechu, and Brassica juncea showed maximum inhibitory effect on A. niger [50]. Spices possess antifungal activity and can be exploited as natural fungitoxicant to control the growth of A. niger [48]. Different concentrations of leaves and stems extract of Moringa olifera showed variable levels of antifungal activity against A. niger mold [51]. Among fourteen spices tested, cardamom, cinnamon, pepper, star anise and stone flower were exhibited inhibitory activity against black mold [14]. Neem leaf extract at 5.0 ml/10 g seeds of onion proved to be effective in improving seed germination and vigor index by reducing pre- and post-emergence mortality and symptoms [9]. Onion ethanol extract (27.5 g/ml), aqueous garlic extract (32.5 g/ ml), and aqueous leek extract (90 g/ml) showed the most inhibitory effects on A. niger [16]. To our knowledge few to negligible studies were conducted on the antimicrobial activity of the four tested plant extracts in the current investigations (Prunus mahaleb, Commiphora myrrha, Syzygium aromaticum, and Panax ginseng). Preliminary investigations on the microbiological activity of the leaves and roots extracts of American ginseng (Panax quinquefolium L.) showed weak activity against bacteria and fungi [20]. It was suggested that the chemical compounds isolated from the hairy roots culture of P. ginseng revealed antimicrobial activity towards Staphylococcus aureus, Bacillus subtilis, Cryptococcus neoformans, and Aspergillus fumigatus [21] The essential oils of Commiphora Africana and C. myrrha show anti-inflammatory, antifungal, antibacterial, and antiviral properties [32,34,52]. The oil of C. molmol causes complete inhibition of sporulation process of A. niger, A. flavus. Penicillium citrinum at concentrations of 0.5-4% [33]. The aqueous and methanolic extracts of C. myrrha and C. molmol have antibacterial effect against Micrococcus lutus, Nisseria siccca, Pseudomonas vulgaris, P. aeroginosa, Klebsiella pneumonia, Escherichia coli, Proteus mirabilis, Enterococcus faecalis, B. subtilis, Salmonella typhi, and Shigella dysenteriae [37]. Ethanolic extract of Prunus mahaleb had antimicrobial activity against Pseudomonas mirabilis, B. anthracis, S. aureus and inhibitory effect to Brucella melitensis, E. coli, and B. licheniformis in low concentrations (0.1-2.0 g/ml) [30]. The extract showed anti-inflammatory property, antibacterial activity at 0.8-6.4 g/ml and antifungal property at 1.6-6.4 g/ml [31,53]. On the other hand, the essential oil of clove (Syxygium aromaticum) was a powerful preservative against Staphylococcus aureus, Streptococcus mutans, Lactobacillus acidophilus, E. coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus sp., A. brasillienses, Saccharomyces serevisiae, and Candida sp. [23,24,27]. The antifungal activity of the essential oil of clove against Fusarium commune, F. moniliforme, F. oxysporum, F. redolens, Aspergillus sp., Mucor sp., Trichoderma rubrum, and Microsporum gypseum at all concentrations was reported [28,29]. Clove acts as larvicidal agent to combat dengue disease in Brazil and other tropical countries [25]. Ethanolic extract of clove showed better antimicrobial property than garlic extract to Gram-positive and Gram-negative food associated bacteria (Bacillus subtilis, M. megaterium, B. polymyxa, B. sphaericus, Staphylococcus aureus, and E. coli) and molds (Penicillium oxalicum, A. flavus, A. luchuensis, Rhizopus stolonifer, Scopulariopsis sp., and Mucor sp.) [23]. These findings stipulate the need for testing the antimicrobial activity of different plant extracts as potential control measures for black mold of onion bulbs. In the present studies, the effects of different concentrations of Prunus mahaleb, Commiphora myrrha (0.5, 1.0, 1.5, and 2%) (Fig. 1), and Syzygium aromaticum, Panax ginseng (0.5, 1.0, 2.0, and 2.5%) (Fig. 2) seed germination/contamination and spore germination of A. niger were investigated. The results showed that these plant extracts significantly ($p<0.05$) inhibited spore germination of A. niger up to 37-97% for Prunus mahaleb, 43-96% (Syzygium aromaticum), 33-88% (Commiphora myrrha), 34-87% (Panax ginseng). These findings indicate their antifungal property against various fungi as suggested by many authors in similar studies using different plant extracts [18,33,48,49,51]. On the other hand, the highest concentration of the four plant extracts (3.0%) did not affect onion seed germination, however, they caused significant ($p<0.05$) reduction in seed contamination by A. niger up to 84% for Syzygium aromaticum, 80% (Commiphora myrrha), 71% (Panax ginseng), and 65% (Prunus mahaleb) (Fig. 3). Similar findings show the antifungal property of different plant extracts against A. niger growth [14,16,48,50]. Essential oils of C. molmol completely inhibited spore germination of A. niger and 90% of A. flavus [33]. The highest growth inhibition of myrrha (74.6%) was against Ulocladium strictum, followed by Trichoderma pseudokoningi (70.6%), whereas the lowest efficiency (12.7%) was reported for U. consortiale 38]. The antifungal activity of the tested plants to different fungal flora was reported by many authors

Table 1: *Percentage of seed germination and contamination of onion seeds by Aspergillus niger.*

Onion Cultivar Local Name	Color	Contamination%	Germination%
Saggai	Red	97	39
Kamleen	Yellow	99	73
Nassi (El Hilo)	White	96	43
Saggai	Red	99	59
Shendi	Red	98	71
Baladi	Red	89	83
Hilaleia	Red	99	77
Kosti	Red	97	67
Wad Taktuk	Red	100	80
Saggai	Red	100	41
Atbara	Red	99	69
Baladi	Yellow	98	51
Omdum	Red	98	39
Abu feraiwa	Yellow	100	41

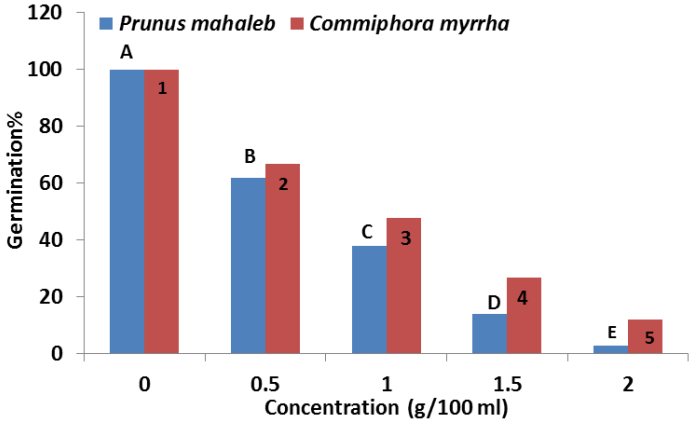

Fig 1: *Effect of different concentrations of Prunus mahaleb and Commiphora myrrha extracts on spore germination of Aspergillus niger*

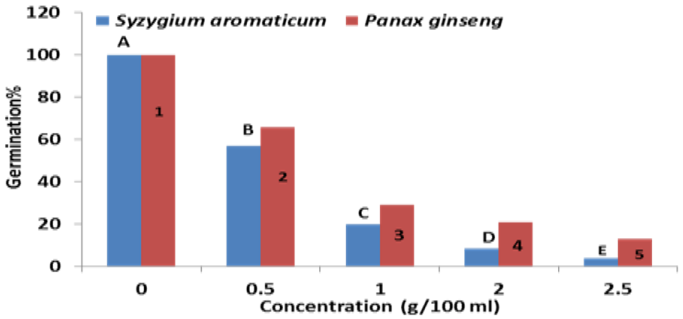

Fig 2: *Effect of different concentrations of Syzygium aromaticum and on Panax ginseng extracts on spore germination of Aspergillus niger*

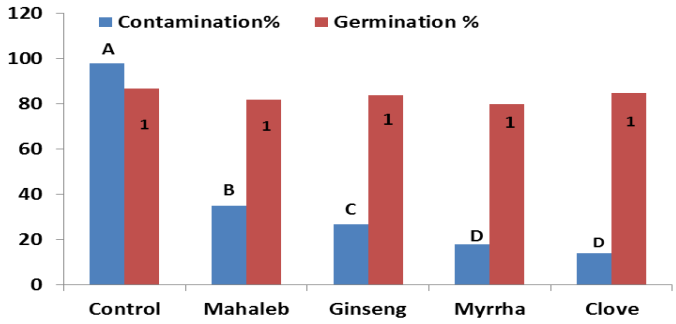

Fig 3: *Effect of the highest concentrations of different plant extracts on seed germination and contamination by Aspergillus niger*

On P. mahaleb [31], P. ginseng [21], C. myrrha [32], and S. aromaticum [22,24,28,29]. In the present investigations, 3% concentration from the selected plant extracts (P. mahaleb, C. myrrha, S. aromaticum, and P. ginseng) evidently reduced the mold growth and development on stored onion bulbs (Fig. 4). Therefore, these plant extract may provide an alternative control measures to synthetic chemicals as concluded by many authors [3,16].

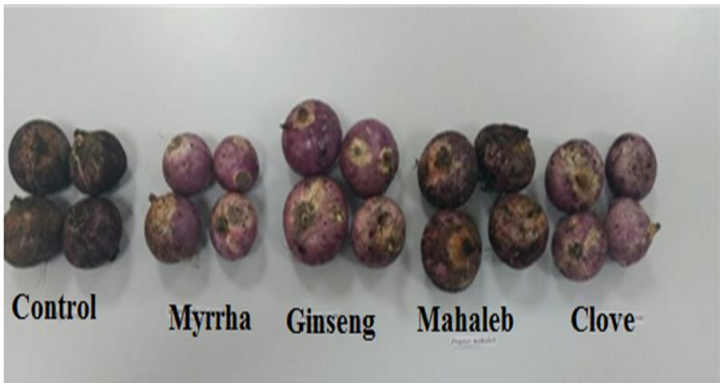

Fig 4: *Effect of different plant extract on Aspergillus niger growth and development on onion bulbs*

Conclusion

Our data indicate that the seed samples of the onion cultivars were highly contaminated by A. niger (89-100%) and displayed low levels of seed germination (39-83%). The different concentrations of the four plant extracts (Syzygium aromaticum, Syzygium aromaticum, Panax ginseng and Prunus mahaleb) significantly reduced seed contamination and spore germination of A. niger as well as mold development on stored bulbs. Therefore, these extracts can be used as natural fungitoxicant agents to control the black mold of onion bulbs. Therefore, the chemical composition and toxicity of the biologically active ingredients need thorough investigations. This will broaden our knowledge on the biological control strategies to combat different plant diseases.

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