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ANTIFUNGAL ACTIVITY OF SOME ESSENTIAL OILS ON FUNGAL GROWTH AND AFLATOXIN PRODUCTION

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ABSTRACT

Pest control is one of the important challenges of the agriculture and food commodity. The contamination of wheat and broad bean with aflatoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus* is the main problem in the export of their product. The inhibition of growth and aflatoxin production by essential oils (EOs) of clove, black cumin, caster bean, opuntia and almond were investigated on both fungi. The antifungal effect of the EOs was observed by determination of growth-rate (mm/day) and antifungal index (%) using reversed Petri-dish method. Aflatoxin production was monitored by thin layer chromatography (TLC). The growth of *A. flavus* and *A. parasiticus* was significantly decreased (P<0,001) by clove and black cumin EOs. TLC results revealed only a highly effect on aflatoxin production: clove, black cumin and opuntia EOs found to decrease the amount of aflatoxin *A. parasiticus* showed almost similar response to the EOs. The EOs tested in this study may be potential antimicrobial compounds for use as food preservatives and anti-aflatoxin agents.

Keywords: Antifungal, Essential oil, Fungal growth, Aflatoxin.

INTRODUCTION

Many fungal species produce mycotoxins contaminating various food and feed [1]. Among them, aflatoxin synthetized by several fungi species from which *Aspergillus flavus* and *A. parasiticus* are the most important ones and which are common in most soils and are usually involved in decay of plant materials. This toxin is produced during plant growth, harvest, storage and food processing. Aflatoxin is extremely hazardous: it has a hepatotoxic, carcinogenic and immunosuppressive effect. it has been classified as a class 1 human carcinogen by the Internal Agency for Research on Cancer [2]. The presence and growth of fungi may cause spoilage of food and mycotoxin production [3]. Therefore, the control of fungi and of aflatoxin biosynthesis is extremely important for agriculture and public health.

Essential oils of aromatic plant were known since ancient times to have some preservative qualities. The essential oils and their constituents have been found effective as antifungal agent [4-6] volatile compounds and the composition of the oil quite often varies between species [7]. It seems that the antifungal and antimicrobial effects are the result of many compounds acting synergistically [8]. There would be negligible chance of development of resistant races of fungi after application of essential oils to fruit and vegetables. As a consequence essential oils are one of the most promising candidate groups of natural compounds for the development of safer antifungal agents [9]. The oil extract of *Nigella sativa* showed *In vitro* and *In vivo* antimicrobial effect against *Candida albicans* [10] and *A. flavus*, *A. niger*, *Drechslera hawiinesis* and *Fusarium* spp [11]. Several authors demonstrated that, most of the essential oils extracted from aromatic plants, have antimicrobial, antifungal effect and/or antioxidant properties. That means they have also a potential to act against mycotoxins-producing fungi [12-14]. The present study was undertaken to investigate the bioactivity potential of some commercial essential oils against *Aspergillus flavus* and *A. parasiticus* isolated from wheat and broad bean at post-harvest in El-Beida- Libya.

MATERIALS AND METHODS

Source of isolations

Associated fungi were isolated from stored wheat and broad bean seeds. The seeds were surface-sterilized in NaOCl for 2 min and rinsed in two changes of sterile distilled water. The seeds were blotted dry in between sterile Whatman No. 1 filter papers and plated on sterile potato Sucrose agar (PSA) at the rate of 10 seeds per plate.

Thirty seeds were plated per crop and incubated at room temperature of 25 ± 1^{o}C. Sub-cultures were made from emerging colonies and pure cultures obtained for subsequent studies.

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Pure cultures of fungi were examined macroscopically and microscopically, and their identification was carried out by using a taxonomic schemes primarily based on morphological characters using the methods given by Singh et al. [15], Filtenborg et al. [16], and Tabuc [17]. Isolates of *A. flavus* and *A. parasiticus* obtained from wheat and broad bean respectively.

Essential oils

Five essential oils- clove (*Eugenia earyophllata*), cumin black (*Nigella sativa*), caster bean (*Ricinus communis*), almond (*Prunus amygdalus*) and opuntia (*Opuntia tuna*) – were used in the experiments. They were purchased from Medicine Importation Company in my city- El-Beida, Libya.

Antifungal activity of Eos

Poisoned Food Technique

Essential oils were subjected to antifungal assay by poisoned food technique following the procedure of Mohana et al. [18]. Potato Sucrose Agar (PSA) medium with 10% concentrations of EO were prepared by adding appropriate quantity of EO to melted medium, followed by manual rotation of Erlenmeyer to disperse the oil in the medium. About 20 ml of the medium were poured into glass Petri dishes (9 cm). The molds grown on PSA for 48 h are transplanted (subculture), using a disc of 5 mm in diameter which carries spores from the anamorphic mold, on the surface of a Petri dish containing the former medium PSA and EO at 10% concentration. Control plates (without essential oil) were inoculated following the same procedure. Plates were incubated at 25°C for 8 days and the mycelial growth was appreciated every day by measuring the average of two perpendicular diameters passing by the middle of the disc [19]. The percentage inhibition (PI) of fungal growth was evaluated by the following equation: PI 0 [1- (d/dc)] × 100 [20], where d is the diameter of growth zone in the test plate, and dc the diameter of growth zone in the control plate (Petri dish without essential oil).

On spores germination

The spore germination test was conducted by using the same oil concentration with the synthetic SMKY broth [21]. A drop of about 0.5 ml was transferred from each solution onto a sterile glass slide and inoculated with 0.1 ml spore suspension (1×10^7 spore/ml). The inoculated slides were placed in the sterile petri dishes lined with moisten sterile filter paper to attain the relative humidity 90- 100% approximately at 28 °C for 20 h. 100 spores were examined and the extent of spore germination assessed by looking for germ tube emergence. The number of spores germinated was scored and reported as a percentage of spore germination.

On mycelia dry weight and aflatoxin production

100 ml of synthetic SMKY medium containing 10% oils n conical flask were inoculated with 1.0 ml spore suspension as above and incubated at 25C°. Seven days later, the mycelial mats were collected, dried and weighted. The resultant fluid was examined for aflatoxin production of both fungi was monitored by thin layer chromatography (TLC) according to method described by Dienner and Davis [22] and Jones [23].

Statistical analysis

All experiments were made in three replicates per treatment. One-way analysis variance (ANOVA) was performed on all the data and least significant differences ($P = 0.05$) between the control and treated samples were determined according to the Duncan's multiple range tests.

RESULTS AND DISCUSSION

Cultural and morphological characteristics of the fungal isolates

On PSA, the fungus formed radial colonies of white airy mycelia, and when the growing fungus is about 1 inch in diameter, yellowish green spores are produced (Figure 1-A.). Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate. The vesicle, phialides, metulae and conidia form the conidial head (Figure 1- B.). The isolate was confirmed by morphological and cultural characters which were Colony: Colour, shape, margins and pigmentation; Mycelium: Colour, shape, septation, Conidia: Colour, shape, size and septation. Based on the cultural and morphological description the fungus was identified as *A. flavus*.

Antifungal activity of clove (*Eugenia earyophllata*), cumin black (*Nigella sativa*), caster bean (*Ricinus communis*), almond (*Prunus amygdalus*) and opuntia (*Opuntia tuna*) oils were determined against two fungi viz., , *A. flavus*, and *A. parasiticus*. These essential oils were tested by agar diffusion plate method caused significant reduction in the growth of above mentioned fungi. The rate of growth reduction was directly proportional to the concentration of tested material in the medium (Fig. 2). The results indicated that only four oils exhibited different degrees of antifungal activities against seed borne fungi. Clove oil showed significant inhibitory effect on fungal growth against all test fungi. It was completely inhibited the growth of both fungi, followed by Cumin oil which allowed to about 18% of growth for both fungi, while Almond and Opuntia oils were less effective, the Caster bean oil was non effective at all. Different studies agreed that Clove and Cumin oils have antimicrobial activities [11,24,25,26]. According to Merory [27] clove oil contain 95% eugenol, the most antimicrobial agent. Misra and Batra [28] found that eugenol was heat resistant, whether both autoclaved and non-autoclaved samples were similarly effective.

Results of the effects of oil on spore germination of both fungi are shown in Figure 3. Clove oil was also the most effective one and gave completely inhibited of germination, followed by B. cumin, then Opuntia, Caster and finally Almond.

Dry weight of mycelial growth and aflatoxin production in broth media containing different oil (10%)

Table 1. Effect of essential oils on aflatoxin production

EO	Aflatoxin concentration (ppm)	
	A. flavus	*A. parasiticus*
Clove	0 c	0 b
Black cumin	0 c	0 b
Caster bean	0.05 c	0.5 b
Opuntia	0 c	0 b
Almond	1 b	0.5 b
Control	100 a	150 a
LSD at 0.05	0.81	0.77

Values are mean 3 replicates. The means followed by same letter in the same column are not significantly different according to ANOVA and Duncan's multiple range tests.

Figure 1. (A) Culture and (B) Conidial head morphology in *A. flavus*.

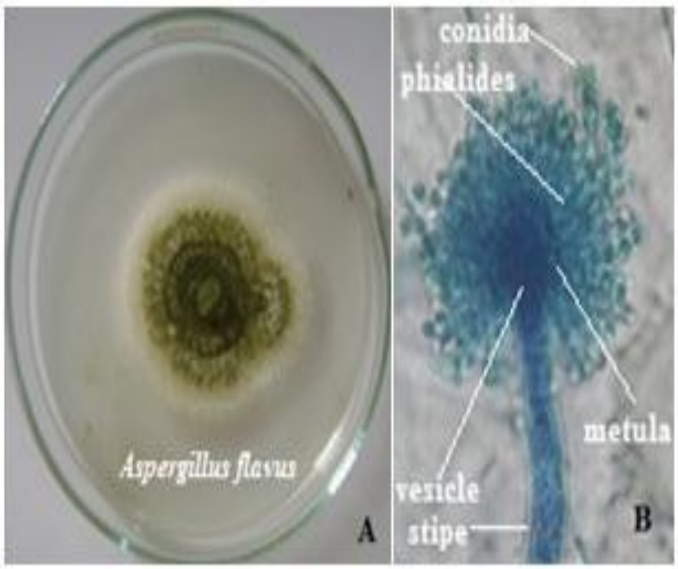


Figure 2. Average growth rate ± SD of *A. flavus* and *A. parasiticus* after treatment with different EOs (10% conc.). Different letters on the top of columns represent significantly different results (P<0.05).

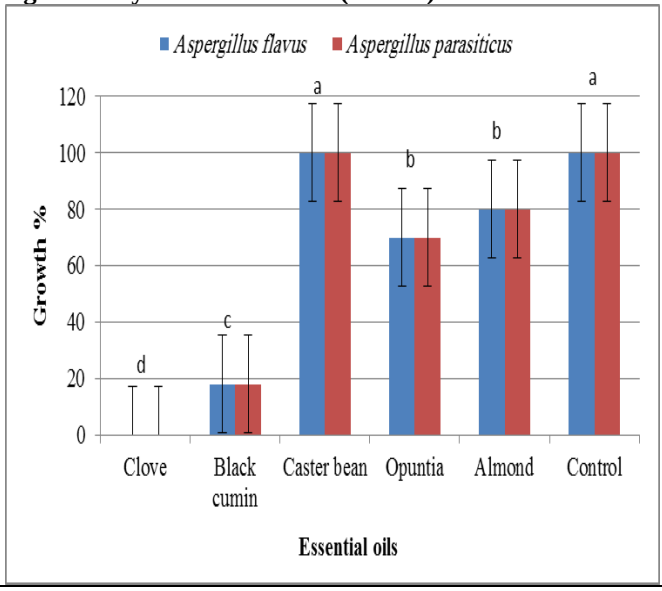


Figure 3. Average spore germination rate ± SD of *A. flavus* and *A. parasiticus* after treatment with different EOs (10% conc.). Different letters on the top of columns represent significantly different results (P<0.05).

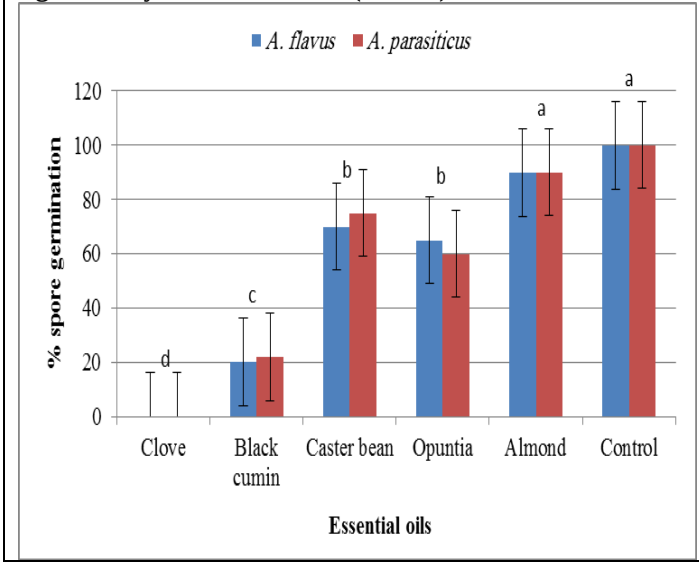
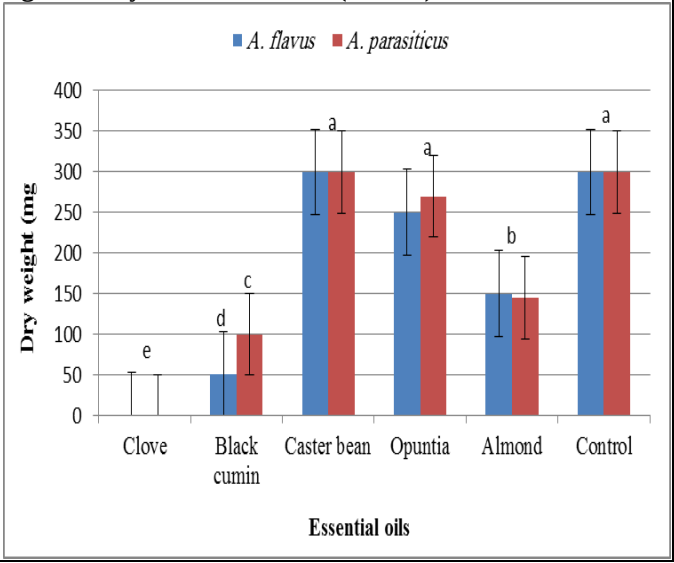


Figure 4. Average dry weight (mg) ± SD of *A. flavus* and *A. parasiticus* after treatment with different EOs (10% conc.). Different letters on the top of columns represent significantly different results (P<0.05).



concentration were showed in Figure 4. Reduction in mycelial dry weight follow the same pattern as that in radial growth and spore germination with Clove and B. cumin oil as the most effective.

Results regarding aflatoxin production can be seen on Table 1. Results show that mycotoxin production of both fungi was highly influenced by the EOs. compared to the control samples there seems to be a difference concentration in aflatoxin ppm in the case of treatment with 10% clove, black cumin and opuntia although oil of opuntia have little effect on fungal growth, it inhibited aflatoxin production completely.

Almond oil gave similar results. Different effect of oils on growth and aflatoxin production were also reported in other studies. El-Shayb and Mabrouk [29] and Osman [30] reported that the extracts of sweet pepper and *hibiscus* sp. inhibited aflatoxin completely while they are non-effective on fungal growth. This study has reported the antifungal properties of five oils tested against two fungi and mycotoxin production. Higher antifungal activity was found in the Eos from clove, black cumin and opuntia. From all essential oils evaluated, only one (*Eugenia earyophllata* essential oil) showed inhibitory activity on both assayed mold strains. In other words, all the mold strains were sensitive to the essential oils of cloves. This work agrees with the work of Pinto et al. [31], who also showed that clove essential oil has wide spectrum antifungal activity. Bonsod and Rai [32] also reported clove's essential oil to have activity against *A. fumigatus* and *A. niger*. Also Shamsuddeen and Sheshe [33] recorded that the effective of clove oil against some molds isolated from foods.

CONCLUSION

The findings of this study, clearly show that the essential oil of clove has antifungal activity and can be used in practical application for the inhibition of mould growth and mycotoxin production in food spoilage fungi. It would be important to establish the actual bioactive component/s and the mode of action of the essential oil against the *Aspergillus* species. Of interest also would be to determine the effect of the oil on mycotoxin production ability of the moulds.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

REFERENCES

1. Naeini A, Ziglari T Shokri H Khosravi AR. Assessment of growth-inhibiting effect of some plant essential oils on different *Fusarium* isolates. *J. Mycol. Medic*, 20, 2010, 174-178.
2. IARC. Some naturally occurring substances: Food items and constituents, heterocyclic aromatic amines and mycotoxins, IARC monographs on the evaluation of carcinogenic risks to humans, 56 International Agency for Research on Cancer, 1993, 489–521.
3. Rasooli I. and Abyaneh M.R. Inhibitory effects of thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. *Food Control*, 15, 2004, 479- 483.
4. Daferera DJ, Zirgas BN. and Polission MG. GC-MS Analysis of essential oil from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *J. Agric. Food. Chem*, 48, 2000, 2576-2581.
5. Sridhar SR, Rajagopal RV. Rajavel R. Masilamani S. and Narasimhan S. Antifungal Activity of some essential oils. *J. Agric. Food. Chem*, 51, 2003, 7596-7599.
6. Karbin S, Rad BA, Arouice H. and Jafarnia S. Antifungal of the essential oils on post-harvest disease agent *Aspergillus flavus*. *Adv. Envir. Biol*, 3(3), 2009, 219 – 225.
7. Mirsh AK and Dubey NK. Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied Environ. Microbiol*, 60, 1994, 1101-1105.
8. Bagamubola CF, Uyttendaele M. and Debevere J. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. *Food Microbiol*, 21, 2004, 33-42.
9. Hadizadeh I, Peivastegan B. and Hamzehzarghani H. Antifungal Activity of Essential Oils from Some Medicinal Plants of Iran against *Alternaria alternata. American Journal of Applied Sciences*, 6(5), 2009, 744-748.
10. Mashhadian NV and Rakhshandeh H. Anti-bacterial and antifungal effects of *Nigella sativa* extracts against *S. aureus, P. aeroginosa* and *C. albicans*. *Pak. J. Med. Sci*, 21(1), 2005, 47-52.
11. Sitara U, Niaz I, Naseem J. and Sultana N. Antifungal effect of essential oils on in vitro growth of pathogenic fungi. *Pak. J. Bot*, 40(1), 2008, 409 – 414.
12. Adjou ES, Sandrine K, Edwige DA, Sohounhloue Dominique CK. and Soumanou MM. Antifungal activity of *Ocimum canum* Essential oil against Toxinogenic Fungi isolated from Peanut Seeds in post-harvest in Benin. *I. Res. J. Biological Sci*, 1(7), 2012, 20-26.
13. Gömöri C, Nacsa-Farkas E, Kerekes EB, Kocsubé S, Vágvölgyi C, and Krisch J. Evaluation of five essential oils for the control of food spoilage and mycotoxin producing fungi. *Acta Biologica Szegediensis*, 57(2), 2013, 113-116.
14. Houinsou FR, Adjou SE, Ahoussi ED, Sohounhloué CK and Soumanou MM. Bioactivity of essential oil from fresh leaves of *Lantana camara* against fungi isolated from stored cowpea in southern Benin. *Int. J. of Biosciences*, 5(1), 2014, 365 – 372.
15. Singh K, Frisvad JC, Thrame U, Mathur SB. An illustrated manuel on identification of some seed- borne *Aspergilli, Fusaria, Penicillia* and their mycotoxines; 1st Edition, Denmark. ISBN 87(7026-3175), 1991, 133.

16. Filtenborg O, Frisvad JC, Thrane U. Moulds in food spoilage. *International Journal of Food Microbiology*, 33, 1996, 85-102.
17. Tabuc C. Flore Fongique de différents substrats et conditions optimales de production des mycotoxines. Thèse de Doctorat, Institut National Polytechinique de Toulouse. 2007, 190.
18. Mohana, DC and Raveesha KA. Anti-mycotic, Anti-biodeteriorative and Anti-aflatoxigenic Potency of 2-hydroxy-4-methoxybenzaldehyde Isolated from *Decalepis hamiltonii* on Fungi Causing Biodeterioration of Maize and Sorghum Grains. *J. Mycol. Plant Pathol*, 40(2), 2010, 197- 206.
19. Yehouenou B, Sessou P, Houinsou R.L, Noudogbessi J.P, Alitonou G.A, Toukourou F. and Sohounhloue D, Chemical composition and Antimicrobial activities of Cinnamomum zeylanicum Blume dry Leaves essential oil against Food-borne Pathogens and Adulterated Microorganisms. *I. Res. J. Biological Sci*, 1(6), 2012, 18-25
20. Kumar VP, Chauhan NS, Padhand H, Rajani M. Search for the antibacterial and antifungal agent from selected Indian Medicinal plants. *Journal of Ethno pharmacology*, 107, 2006, 182-188.
21. Abdel-Rahim AM and Arbab HA. Factors effecting conidiospre germination in *Aspergillus niger*. *Mycopathol*, 89, 1985, 75 -79.
22. Diener UL. And Davis DN. Aflatoxin production by isolates of *Aspergillus flavus*. *Phytopathology*, 56, 1966, 1390 – 1393.
23. Jones BD. Methods of aflatoxin analysis. *Report of the Tropical Products Institute*, 24, 1957, 35 -39.
24. Singh R. and Rai B. Antifungal potential of some higher plants against *Fusarium* causing with diseases of *Cajanis Cajan*. *Microbios*, 102, 2000, 165-173.
25. Dusanee T, Suvathi Y, Sripakdee PS, Patthanawanitchai O. and Charoensettasilp S. Effect of essential oil on growth of *Aspergillus flavus*. *Sci. Tech. J*, 7(01), 2007, 1-7.
26. Shirurkar D and Wahegaonkar KN. Antifungal activity of selected plant derived oils and some fungicides against seed borne fungi of maize. *European Journal of Experimental Biology*, 2(5), 2012, 1693-1696.
27. Merory J. Food flavorings composition, Manufacture and Use. Avi. Pubishing Company, Westport, 1960, 114.
28. Mishra N. and Batra S. Efficiency of essential oil of *Cinnamomum tamala* Nees and Eidam against *Aspergillus flavus* NRRL 3251 and *A. parasiticus* NRRL 2999 producing mycotoxins in stored seed at groundnuts. *Ind. Perf. (Abst.)*, 31(4), 1987, 332 – 334.
29. El-Shayeb NM. And Mabrouk SS. *Nutrition Reports International*, 29, 1984, 273- 282.
30. Osman NA. Survey of some cereal grains and legumes for the presence of aflatoxin in the Sudan. M. Sc. Thesis, University of Khartoum. 1986, 194.
31. Pinto N, Silva LV, Cavaleiro C, Salgueiro L. Antifungal activity of the clove essential oil from *Syzygium aromaticum* on *Candida*, *Aspergillus* and *Dermatophyte* species. *J. of Med. Microbiol*, 58, 2009, 1454–1462.
32. Bonsod S, Rai M. Antifungal Activity of Essential Oils from Indian Medicinal Plants against Human Pathogenic *Aspergillus fumigatus* and *A. niger*. *World J. of Med. Sci*, 3(2), 2008, 81-88.
33. Shamsuddeen U. and Sheshe KI. Inhibitory activity of clove, moringa and neem essential oils on the growth of some molds isolated from foods. *Sky Journal of Microbiology Research*, 2(3), 2014, 018 – 021.