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Efficacy of Essential oil of *Lippia alba* Against Fungi Causing Post-Harvest Diseases of Some Fruits

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ABSTRACT: The essential oil of *Lippia alba* is reported as an antifungal against human pathogenic microorganisms but few articles report its use as an alternative to synthetic fungicides on green mould control. The objective of this study was to determine chemical characteristics of *L. alba* essential oil and its antifungal activity against green molds as an alternative to synthetic fungicides. Essential oil was extracted by Clevenger hydrodistillation, characterized by GC-MS analysis, and the structure of the main compounds confirmed by ^{1}H and ^{13}C-NMR spectroscopy. Microdilution assays evaluated the essential oil minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC). Commercial fungicides Ketoconazole and Bifonazole were used as control. Essential oil yield is of 0.15% and the major components are neral (33.32%) and geranial (50.94%). The *L. alba* essential oil has MIC of 0.300–1.250 mg/mL and MFC of 0.600–1.250 mg/mL. Ketoconazole and Bifonazole show MIC ranging from 0.025–0.500 to 0.100–0.200 mg/mL, and MFC ranging from 0.250–0.100 to 0.200–0.250 mg/mL, respectively. *L. alba* essential oil is classified as citral type and the results indicate that it is a potential alternative to synthetic fungicides and controls post-harvest diseases of some fruits.

KEYWORDS: essential oil ,*Lippia alba* , fungi, antifungal, post harvest diseases, fruits, efficacy

I. INTRODUCTION

Lippia alba (Mill.) N.E. Br. ex Britton & P. Wilson (Verbenaceae) is an aromatic plant widely used all over South and Central America for different purposes. This family comprises over 175 genera and 2,800 species in Africa, Latin America (25) and India (6, 3). Several papers have presented ethnopharmacological studies dealing with *L. alba* as sedative, antidepressant and analgesic properties (18). The essential oil of *L. alba* also has many applications such as stomachic, anti-spasmotic, digestive, anti-hemorrhoidal and anti-asthmatic (18). Different biological activities such as cytotoxic, antifungal, antibacterial, antiviral and anti-inflammatory, have been identified in essential oils or extracts of *L. alba* (5, 11, 19, 23).

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L. alba

Mesa-Arango *et al*. (25) reported two chemotypes, citral and carvone but three chemotypes, citral, carvone and linalool for the same species. It was stated that the main constituents of *L. alba* essential oil were geranial varying from 22.21% to 33.98% and neral varying from 14.20% to 25.82%. Although *L. alba* essential oil is well described, its chemotypes and constituents may vary according to the environment. Thus, it is important to determine the chemical characterization of *L. alba* essential oil in order to identify the components that have antifungal activity and controls post-harvest diseases of some fruits.[1,2]

The antifungal activity of the essential oils of *L. alba* against human pathogenic fungi such as *Candida albicans*, *Candida guilliermondii, Candida parapsilosis*, *Candida neoformans*, *Trichophyton rubrum* and *Fonsecaea pedrosoi* has been previously demonstrated for the citral and myrcene-citral chemotypes (26). *L. alba* essential oil and two of its major components were evaluated for fungitoxicity and anti-aflatoxigenicity against *Aspergillus flavus* (32). The antifungal activity was evaluated against *C. parapsilosis*, *Candida krusei*, *A. flavus* and *Aspergillus fumigatus* strains (25). Antifungal screening was carried out also against *Saccharomyces cerevisiae*, *A. flavus*, *Aspergillus niger and C. albicans* (3,4).

Although many studies reported the antifungal activity of *L. alba* essential oils against human pathogenic microorganisms, few articles were about *Fusarium*, *Aspergillus* and *Penicillium* genera (5). These fungi are well known as causal agents of food-borne diseases and food spoilage, which increase the costs of food production and health care in the world (6,7). In addition, the genera *Trichoderma*, *Aspergillus* and *Penicillium*, known as green moulds, occur on mushroom production when the composting is not correctly prepared and/or does not become selective enough (8). Those fungi can spread very rapidly competing for carbohydrates in the substrate at the time of spawning, or in the casing layer, and some of them produce toxins that can damage mushroom tissue (9,10). The genus *Trichoderma* is the most common contaminant on mushroom cultivations and facilities causing enormous economic losses around the world (11,12). Many sanitary procedures were adopted in mushroom farms to control *Trichoderma* sp and other green molds (15) and also on spawn production or spawning procedures where synthetic fungicides are used on mushroom cultivation. Although the fungicides that are commonly used in cultivation are very effective and inexpensive, it has been suggested that they leave residual toxicity that may cause side effects, including carcinogenesis and teratogenesis (13,14). Most of these synthetic fungicides have been restricted in several countries since the early 1960's.

Despite of the potential use of essential oils on fungus control, there are no reports about the essential oil toxic effects on basidiomycete development or the use on mushroom production. Thus, new studies should be done to evaluate the viability of spraying essential oil solutions on the surface of casing layers where green molds are very common. Also, microbicidal essential oils are generally considered less harmful than synthetic chemicals and are being used on other organic cultures (16). An additional advantage of essential oils is their volatile nature, which implies on low or no residues after treatment and low environmental impact.[17]

Based on alternatives for organic production with natural substances to control undesirable fungi, the objective of this study was to determine the chemical characteristics of *L. alba* essential oil and its antifungal activity against green molds as an alternative to synthetic fungicides and for control of post-harvest diseases of some fruits.

II. DISCUSSION

Food scarcity is one of the important major problems faced by several countries. It is reported that nearly 1 billion people are challenged by severe hunger in these nations of which 10% actually die from hunger-related complications. A substantial part of this hunger problem stems from inadequate agricultural storage and produce preservation from microbes-induced spoilages . The most important losses in agricultural productions which involve the greatest costs on the farm economy occur by post harvest diseases. It is estimated that 10 to 40% losses nation of agricultural produce occur due to post harvest diseases worldwide. Losses are more severe in developing

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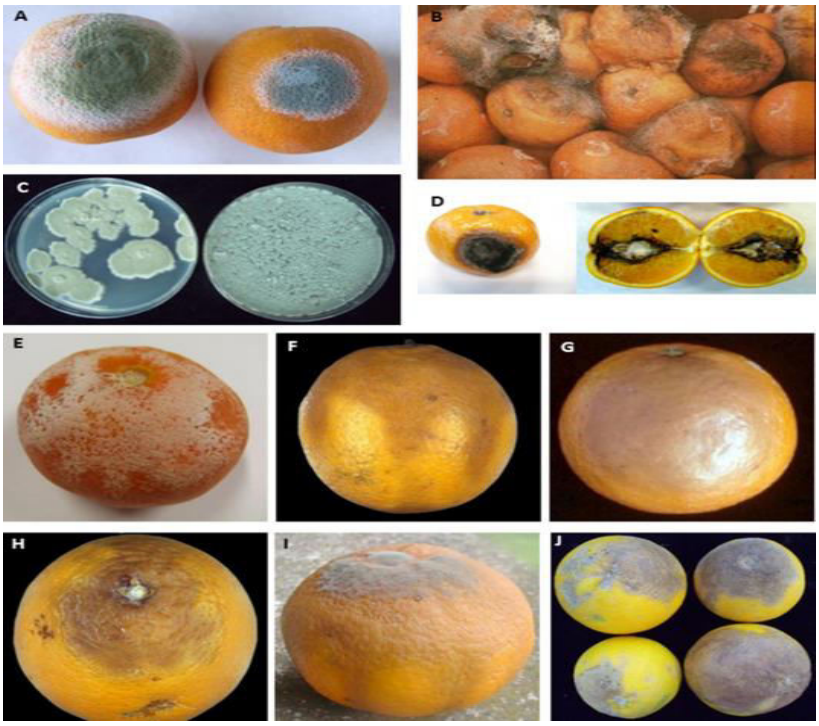
than developed nations of the world . Post harvest activities include harvesting, handling, storage, processing, packaging, transportation and marketing [18,19] .These post harvest losses are caused by the disease which occurs on fruits and vegetables. Post-harvest diseases destroy 10-30 % of the total yield of crops and in some perishable crops especially in developing countries; they destroy more than 30% of the crop yield . Fresh fruits and vegetables are an important source of nutrients in human diet. However, these are perishable after harvest and the mechanical damages during and after the harvest can result in their spoilage by microorganisms such as fungi (*Alternaria, Aspergillus, Botrytis, Ceratocystis, Cladosporium, Colletotrichum, Fusarium, Geotrichum, Lasiodiplodia, Monilinia, Mucor, Penicillium, Pestalotiopsis, Phoma, Phomopsis, Phytophthora*, and *Rhizopus*), bacteria (*Erwinia, Pseudomonas, Xanthomonas, Acetobacter*, and *Enterobacter*), and yeast (*Candida* and *Saccharomyces*) .This is one of the main causes of global economic loss in the agricultural sector.[20,21]

There are several causes of post-harvest losses in fruits and vegetables, and microbial infections are responsible for the greatest losses that occur during the transport, storage, and sale of these products. Chemical control is the most used method to control post-harvest diseases in fruits and vegetables by directly applying synthetic fungicides to the product to be consumed. However, the indiscriminate use of fungicides may be associated with serious toxicity problems in humans and environmental imbalance. Mycofumigation, which is the use of volatile antimicrobial organic compounds produced by fungi to inhibit microbial growth, has become a promising alternative for controlling phytopathogenic fungi associated with post-harvest diseases in fruits and vegetables. The technique has some advantages relative to traditional disease control methods, for example, it does not require direct contact between the antagonist and the plant product, the antimicrobial volatiles diffuse easily in closed environments, they do not leave residues on the plant product to be consumed, and most of the antimicrobial volatile mixtures exhibit bioactivity against a wide range of microorganisms, including many phytopathogens associated with post-harvest diseases. This review highlights mycofumigation as a method for controlling post-harvest diseases in fruits and vegetables, emphasizing the effects of volatile compounds on phytopathogenic fungi and their potential to be applied during the transport and storage of fresh fruits and vegetables.[22]



Post harvest diseases of fruits by fungi

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III. RESULTS

The use of essential oils from different sources and their constituents on the control of postharvest decay and overall quality preservation of fresh fruit and vegetables. An overview on the most significant postharvest diseases and their impact, as well as on the mainly used synthetic chemicals and their side-effects has been included. Emphasis has been on the sources of essential oils and their constituents studied up to now, and their effects on controlling postharvest diseases, either in vitro or in vivo, and their effect on overall quality and storage life of fresh commodities.

Although alternative methods are being researched to control postharvest decay during storage, natural plant products such as EOs of *Lippia alba* are gaining popularity and drawing the attention of researchers globally due to their biodegradable, eco-friendly, economical and safety characteristics. The EOs reported in various studies exhibit antimicrobial, allelopathic, antioxidant and bio regulatory properties

Postharvest life of the horticultural produce, while maintaining the overall fruit quality, nutritional compounds and consumer acceptance of the produce. The major advantage of using EOs of *Lippia alba* in the polymeric matrices of coatings is that they help to slow down the diffusion rate of antimicrobial agents, leaving higher concentrations of active compounds in contact with the fruit surface[23]

IV. CONCLUSIONS

EOs of *Lippia alba* confirmed an expected promising fungicidal activity constituting a potential source for the development of biofungicide. Moreover, EOs preserved the quality parameters in treated strawberry fruits. TP and POD of fruits increased in the fruit treated with *Lippia alba* EOs. Reduction of fruit decay development and DS in the fruits treated with the EOs of *Lippia alba* might be associated with an increase in phenol content and the activity of defense-related enzymes such as peroxidase. In addition, results showed that EOs were capable of maintaining the sensory quality such as fruit taste, flavor, and overall evaluation (except fruit odor) during the postharvest period. These results confirmed that *Lippia alba* EOs can be utilized as biofungicides in the protection of strawberry instead of chemical fungicides with the lowest negative effects on the physicochemical, qualitative, and sensory properties of fresh fruits. However, the feasibility of use as fungicide must be discussed in a wider view before reaching the conclusion of having identified a promising alternative to chemical pesticides. In particular, the availability of sufficient quantities at a reasonable cost and the absence of negative effect on the quality of the treated fruits must be carefully considered in the development of a botanical fungicide.[23]

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