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Fungitoxic and insecticidal properties of essential oils of *Chenopodium ambrasioides* against *Aspergillus flavus* and *Sitophilus oryzae*

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ABSTRACT: *Chenopodium ambrosioides*, known as Jesuit's tea, Mexican-tea,[2] *payqu (paico)*, *epazote*, *mastruz*, or *herba sanctæ Mariæ*, is an annual or short-lived perennial herb native to Central America, South America, and southern Mexico.It is an annual or short-lived perennial plant (herb), growing to 1.2 m (3 ft 11 in) tall, irregularly branched, with oblong-lanceolate leaves up to 12 cm (4+½ in) long. The flowers are small and green, produced in a branched panicle at the apex of the stem.

As well as in its native areas, it is grown in warm temperate to subtropical areas of Europe and the United States (Missouri, New England, Eastern United States),[3] sometimes becoming an invasive weed. The species was described in 1753 by Carl Linnaeus as *Chenopodium ambrosioides*.[4] Some researchers treated it as a highly polymorphic species with several subspecies. Today these are considered as their own species of genus *Dysphania* (e.g. American wormseed, *Chenopodium ambrosioides* var. *anthelminticum* is now accepted as *Dysphania anthelmintica*).[5][6]

The generic name *Dysphania* traditionally was applied in the 1930s to some species endemic to Australia. Placement and rank of this taxon have ranged from a mere section in *Chenopodium* to the sole genus of a separate family Dysphaniaceae, or a representative of Illicebraceae. The close affinity of *Dysphania* to "glandular" species of *Chenopodium sensu lato* is now evident.[7]

The species name refers to the resemblance to plants of the Ambrosia genus, in a different family.

Ideally collected before going to seed, *D. ambrosioides* is used as a leaf vegetable, herb, and herbal tea[8] for its pungent flavor. Raw, it has a resinous, medicinal pungency, similar to oregano, anise, fennel, or even tarragon, but stronger. The fragrance of *D. ambrosioides* is strong and unique.[8] A common analogy is to turpentine or creosote. It has also been compared to citrus, savory, and mint.

Although it is traditionally used with black beans for flavor and its antiflatulent properties,[8] it is also sometimes used to flavor other traditional Mexican dishes: it can be used to season quesadillas and sopes (especially those containing huitlacoche), soups, mole de olla, tamales with cheese and chili peppers, chilaquiles, eggs and potatoes, and enchiladas. It is often used as an herb in fried white rice, and it is an important ingredient for making the green salsa for chilaquiles.

KEYWORDS: Fungitoxic , insecticidal properties , essential oils , *Chenopodium ambrasioides* , *Aspergillus flavus* , *Sitophilus oryzae*

I. INTRODUCTION

Humans have died from overdoses of *D. ambrosioides* essential oils (attributed to the ascaridole content). Symptoms include severe gastroenteritis with pain, vomiting, and diarrhea.[9] The essential oils of *D. ambrosioides* contain terpene compounds, some of which have natural pesticide capabilities.[10] The compound ascaridole in epazote inhibits the growth of nearby species, so it is best to grow it at a distance from other

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plants.[11] *Dysphania ambrosioides* not only contains terpene compounds, but it also delivers partial protection to nearby plants simply by masking their scent to some insects, making it a useful companion plant. Its small flowers may also attract some predatory wasps and flies. Epazote contains oil of chenopodium, which Merriam-Webster defines as "a colorless or pale yellow toxic essential oil of unpleasant odor and taste, … formerly used as an anthelmintic".[12]

Chenopodium ambrosioides

Epazote essential oil contains ascaridole (up to 70%), limonene, p-cymene, and smaller amounts of numerous other monoterpenes and monoterpene derivatives (α-pinene, myrcene, terpinene, thymol, camphor and trans-isocarveol). Ascaridole (1,4-peroxido-p-menth-2-ene) is rather an uncommon constituent of spices; another plant owing much of its character to this monoterpene peroxide is boldo. Ascaridole is slightly toxic and has a pungent, not very pleasant flavor. In pure form, ascaridole decomposes violently upon heating, but this is relatively weak in regards to energy release, since it does not destroy the entire molecule.[13] Ascaridole content is lower in epazote from Mexico than in epazote grown in Europe or Asia.[14]

Leaves of *C. ambrosioides* were dried naturally on laboratory benches at room temperature (26–28°C) for 5 days until they were crisp dry. The dried material was stored in a refrigerator until needed. The powder was obtained by grinding the dried leaves in a coffee grinder. The chemical constituent analysis of the leaf oil yielded five main compounds representing 96% of the oil. The compounds were identified as *α*-terpinen (37.6%), cymol (*p*-cymen) (50.0%), *cis*- *β*-farnesen (1.4%), ascaridole (3.5%) and carvacrol (3.3%). The essential oil was found to be antifungal, and insect pests.[15,16]

II. DISCUSSION

Aspergillus flavus is a saprotrophic and pathogenic[1] fungus with a cosmopolitan distribution.[2] It is best known for its colonization of cereal grains, legumes, and tree nuts. Postharvest rot typically develops during harvest, storage, and/or transit. Its specific name *flavus* derives from the Latin meaning yellow, a reference to the frequently observed colour of the spores. *A. flavus* infections can occur while hosts are still in the field (preharvest), but often show no

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symptoms (dormancy) until postharvest storage or transport. In addition to causing preharvest and postharvest infections, many strains produce significant quantities of toxic compounds known as mycotoxins, which, when consumed, are toxic to mammals.[3] *A. flavus* is also an opportunistic human and animal pathogen, causing aspergillosis in immunocompromised individuals.[4] *Aspergillus flavus* is found globally as a saprophyte in soils and causes disease on many important agriculture crops. Common hosts of the pathogen are cereal grains, legumes, and tree nuts[17,18] Specifically, *A. flavus* infection causes ear rot in corn and yellow mold in peanuts either before or after harvest.[4] Infection can be present in the field, preharvest, postharvest, during storage, and during transit. It is common for the pathogen to originate while host crops are still in the field; however, symptoms and signs of the pathogen are often unseen. *A. flavus* has the potential to infect seedlings by sporulation on injured seeds. In grains, the pathogen can invade seed embryos and cause infection, which decreases germination and can lead to infected seeds planted in the field. The pathogen can also discolor embryos, damage seedlings, and kill seedlings, which reduces grade and price of the grains. The incidence of *A. flavus* infection increases in the presence of insects and any type of stress on the host in the field as a result of damage. Stresses include stalk rot, drought, severe leaf damage, and/or less than ideal storage conditions.[3] Generally, excessive moisture conditions and high temperatures of storage grains and legumes increase the occurrence of *A. flavus* aflatoxin production.[4] In mammals, the pathogen can cause liver cancer through consumption of contaminated feed or aspergillosis through invasive growth.[4] *Aspergillus flavus* colonies are commonly powdery masses of yellowish-green spores on the upper surface and reddish-gold on the lower surface. In both grains and legumes, infection is minimized to small areas, and discoloration and dullness of affected areas is often seen. Growth is rapid and colonies appear downy or powdery in texture.[5]

Hyphal growth usually occurs by thread-like branching and produces mycelia. Hyphae are septate and hyaline. Once established, the mycelium secretes degradative enzymes or proteins which can break down complex nutrients (food). Individual hyphae strands are not typically seen by the unaided eye; however, conidia producing thick mycelial mats are often seen. The conidiospores are asexual spores produced by *A. flavus* during reproduction.[5][6][7]

The conidiophores of *A. flavus* are rough and colorless. Phialides are both uniseriate (arranged in one row) and biseriate.[5]

Recently, *Petromyces* was identified as the sexual reproductive stage of *A. flavus*, where the ascospores develop within sclerotia.[4] The sexual state of this heterothallic fungus arises when strains of opposite mating type are cultured together.[8] Sexual reproduction occurs between sexually compatible strains belonging to different vegetative compatibility groups.

Aspergillus flavus is complex in its morphology and can be classified into two groups based on the size of sclerotia produced. Group I consists of L strains with sclerotia greater than 400 µm in diameter. Group II consists of S strains with sclerotia less than 400 µm in diameter. Both L and S strains can produce the two most common aflatoxins (B1 and B2). Unique to the S strains is the production of aflatoxin G1 and G2 which typically are not produced by *A. flavus*.[4] The L strain is more aggressive than the S strain, but produces more less aflatoxin. The L strain also has a more acidic homoeostatic point and produces less sclerotia than the S strain under more limiting conditions.[9]

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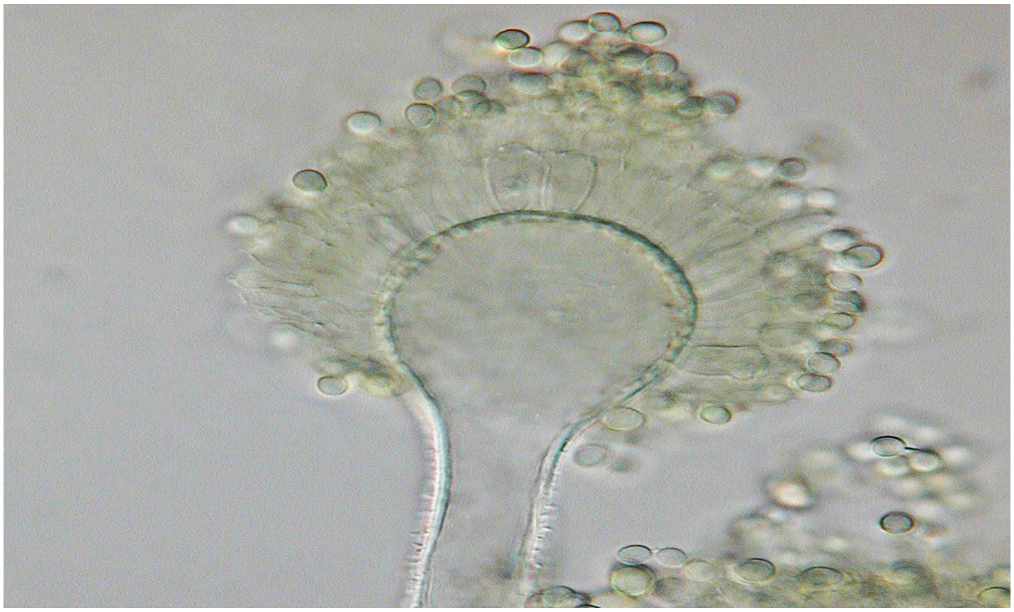

Morphology of *Aspergillus flavus*

To ensure grains and legumes remain free of *A. flavus* infection, certain conditions must be incorporated before, during, and after harvest. Moisture levels should be kept below 11.5%. Temperature in storage units should be kept as low as possible since the pathogen is unable to grow below 5 °C. The low temperature facilitates slower respiration and prevents moisture increase. Fumigants are used to decrease the occurrence and persistence of insects and mites, which aids the rapid growth of the pathogen. Sanitary practices including, removing old and unripe seeds, exclusion of damaged and broken seeds, and overall cleanliness assist in minimizing the colonization and spread of the pathogen.[3]

The most common management practice for grains and legumes is the use of aeration systems. Air is pushed through the storage bins at low flow rates, which removes excess moisture and heat. Regulation of air flow allows the moisture content in harvested products to remain at a constant level and decreases the temperature within the bins. Temperature levels can decrease enough so insects and mites are dormant, [19,20] which reduces rapid growth of the pathogen.[3]

Some environmental control practice have been explored to aid in the reduction of *A. flavus* infection. Resistant crop lines have shown little to no protection against unfavorable environmental conditions. However, good irrigation practices aid in the reduction of stress brought upon by drought, which in turn, reduces the likelihood of pathogen infection. Some research has been done in identifying particular plant proteins, both pathogen-related and drought-resistant proteins, that defend against *A. flavus* entry.[4]

To protect tree nuts and corn plants affected by *A. flavus*, scientists of the Agricultural Research Service found that treating these plants with the yeast *Pichia anomala* reduced the growth of *A. flavus*. The study showed that treating pistachio trees with *P. anomala* inhibited the growth of *A. flavus* up to 97% when compared to untreated trees.[14] The yeast successfully competes with *A. flavus* for space and nutrients, ultimately limiting its growth.[15]

Essential oils of Chenopodium inhibit growth.[21]

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III. RESULTS

The rice weevil (*Sitophilus oryzae*) is a stored product pest which attacks seeds of several crops, including wheat, rice, and maize. The adults are usually between 3 and 4.6 mm long, with a long snout. The body color appears to be brown/black, but on close examination, four orange/red spots are arranged in a cross on the wing covers. It is easily confused with the similar looking maize weevil. The maize weevil is typically somewhat larger than the rice weevil, but rice weevils as large as the largest maize weevils and maize weevils nearly as small as the smallest rice weevils have been found. Some external features can be used to differentiate the vast majority of adults, but the only reliable features are on the genitalia (see table below). Both species can hybridize. The genitalic structure of hybrids is unknown.[2][3][4][5] Control of weevils involves locating and removing all potentially infested food sources and involving application of chenopodium oil. [22]

Sitophilus oryzae

With inaccessibility to synthetic pesticides, farmers are left with the choice of using locally available plant based pesticides. For this reason, we tested the insecticidal potentials of essential oils (EOs) of *Chenopodium ambrosioides* and their binary combinations being antifungal against *Aspergillus flavus* and insecticidal rice weevil on stored rice. Mortality, progeny inhibition, repellence and damage were tested. Pesticide characteristics of both essential oils were dose-dependent, 200 µL/kg of all the combinations caused at least 80% mortality within 14 days of storage while the 50:50 combination completely inhibited progeny production. Moreover, 8 µL of all the EO were repellent to the weevils. The 50:50 binary combination was the most active in all the tests carried out. Pesticidal interactions between the oils in different combination were mostly additive and synergistic. There was also a good control of insect population increase and grain damage after six months of storage. Therefore both EOs can be recommended for the control of Rice against fungus and weevils. [23]

IV. CONCLUSIONS

The chemical composition of essential oil isolated by hydrodistillation from the aerial parts of Chenopodium was analyzed by GC-MS. Carvacrol (87.0%), p-cymene (2.0%), linalool acetate (1.7%), borneol (1.6%) and beta-caryophyllene (1.3%) were found to be as main constituents. Antifungal, phytotoxic and insecticidal activities of the oil and its aromatic monoterpene constituents, carvacrol, p-cymene and thymol were also determined. The antifungal assays showed that this oil, carvacrol and thymol completely inhibited mycelial growth of 17 phytopathogenic fungi and their antifungal effects were higher than commercial fungicide, benomyl. However, p-cymene possessed lower antifungal activity. The oil, carvacrol and thymol completely inhibited the seed germination and seedling growth of certain weeds. However, p-cymene did not show any phytotoxic effect. Furthermore, the oil showed 68.3% and 36.7% mortality against *Sitophilus oryzae*, respectively. The findings of the present study suggest that antifungal and

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herbicidal properties of the oil can be attributed to its major component, carvacrol, and these agents have a potential to be used as fungicide, herbicide as well as insecticide.[23]

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