THE EFFECT OF ACAPULCO (*CASSIA ALATA*) LEAF EXCTRACTS ON THE MANGO PATHOGEN *COLLETOTRICHUM GLOEOSPORIOIDES*

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by

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ABSTRACT

The fungus, *Colletotrichum gloeosporioides,* which causes anthracnose, is known to infect a wide variety of hosts. Mango anthracnose is the most widespread and serious pre- and postharvest disease of mango.

Medicinal plants serve as alternatives in treating various diseases in rural areas. Acapulco's (*Cassia alata*) leaves or sap are used to treat fungal infections such as ringworm. They contain a fungicide, chrysophanic acid. The effectiveness of this plant against skin diseases is confirmed by modern scientific studies.

This study aimed to determine the antifungal reaction of *Colletotrichum gloeosporioides* to the Acapulco (*Cassia alata*) leaf extracts.

Based on the attempts, it was observed that the extracts did not inhibit the growth of *Colletotrichum gloeosporioides* although the positive control, Armor, inhibited its growth.However no substantive result could be achieved because of the presence of contaminants.

Maximum sterility should be ensured in order to prevent contamination. Different concentrations may be used in the next experiments to determine whether other concentrations of the methanol extracts of Acapulco (*Cassia alata)* can inhibit the growth of the fungus. The solvent used can also be changed because other chemicals can be substituted to dissolve the leaf extracts of Acapulco (*Cassia alata*).

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INTRODUCTION

A. Background of the Study

The organisms of the fungal lineage include mushrooms, rusts, smuts, puffballs, truffles, morels, molds, and yeasts, as well as many less well-known organisms (Alexopoulos et al., 1996). About 70,000 species of fungi have been described; however, some estimates of total numbers suggest that 1.5 million species may exist (Hawksworth, 1991; Hawksworth et al., 1995).

Some fungi have important contributions by providing numerous drugs and food. Others cause a number of plant and animal diseases. Plant diseases caused by fungi include rust, smuts, leaf, root, and stem rots, and may cause severe damage to crops and affect the livelihood of the people.

The fungus, *Colletotrichum gloeosporioides,* which causes anthracnose, is known to infect a wide variety of hosts. This disease is regularly seen in the field on ripe or overripe fruits, but they are not a serious problem with unrefrigerated fruit sold in the local markets. It is most important on fruits that are refrigerated and exported to overseas markets.

Mango anthracnose is the most widespread and serious pre- and postharvest disease of mango (Dodd et al., 1991). Although infection of blossom or young fruit can result in failure to produce fruit, the most damaging phase of the disease begins as a quiescent infection, when the fruit is in the preclimacteric phase of development. Growth of the pathogen is resumed only after harvest when the fruit starts to ripen and postharvest anthracnose develops.

Anthracnose is largely a seed borne disease, but may be spread on trash, machinery or by animals and birds. Seedlings that emerge from infected seed can develop lesions on the roothypocotyl or cotyledons. Lesions are generally oval shaped, pink to beige and up to2 cm long. These cause the stem to bend and may progress to infect the pods and seeds.

In controlling anthracnose, resistant plant varieties are chosen when possible and western grown seeds, which have not been exposed to the disease are used. To avoid the spread of this disease, all garden tools are disinfected (one part bleach to 4 parts water) after use. Infected leaves,

fruit, or stems are not used as composts and gardens are thoroughly cleaned. Sulfur or copper powders/sprays are applied weekly to infected plants starting when the disease is first noticed and continuing throughout the growing season. These organic fungicides will not kill the disease, but prevent the spores from germinating. Seeds may also be treated prior to planting.

Medicinal plants serve as alternatives in treating various diseases in rural areas. Since these plants are widely used, many scientists are conducting studies on how these medicinal plants can be of use in other areas, especially agriculture.

Acapulco (*Cassia alata*) is a shrub that grows wild on Mt. Banahaw. It is an erect tropical, annual herb with leathery compounded leaves. It grows up to 6' tall. This perennial shrub has erect waxy yellow spikes that resemble fat candles before the individual blossoms open. The large leaves are bilateral - symmetrical opposed and fold together at night. The fruit is a pod, while the seeds are small and square. Acapulco is indigenous to Suriname and it is found in secondary vegetation or along riverbanks or moist and even wet spots. It is also a host plant to many species of sulphur caterpillars, included the orange barred sulphure. Acapulco is a fast grower and will flower in the first year.

The leaves or sap are used to treat fungal infections such as ringworm. They contain a fungicide, chrysophanic acid. Because of its anti-fungal properties, it is a common ingredient in soaps, shampoos and lotions in the Philippines. The effectiveness of this plant against skin diseases is confirmed by modern scientific studies.

Other chemicals contained in the plant includes saponin which acts as a laxative and expels intestinal parasites. In Africa, the boiled leaves are used to treat high-blood pressure. In South America, besides skin diseases, it is also used to treat a wide range of ailments from stomach problems, fever, asthma to snake bite and venereal diseases (syphilis, gonorrhoea).

B. Statement of the Problem

Antifungal reaction of *Colletotrichum gloeosporioides* to the Acapulco (*Cassia alata*) leaf extracts.

C. Objectives of the Study:

C.A. General Objectives

C.A.1. To determine the antifungal reaction of *Colletotrichum gloeosporioides* to the Acapulco (*Cassia alata*) leaf extracts

C.B.Specific Objectives

C.B.1. To apply the methanol extracts of Acapulco (*Cassia alata*) leaves with the concentrations of 300 mL, 240 mL, and 180 mL

C.B.2. To determine the zone of inhibition of *Colletotrichum gloeosporioides* after exposure to three concentrations of 300 mL, 240 mL, and 180 mL after 7 days of incubation

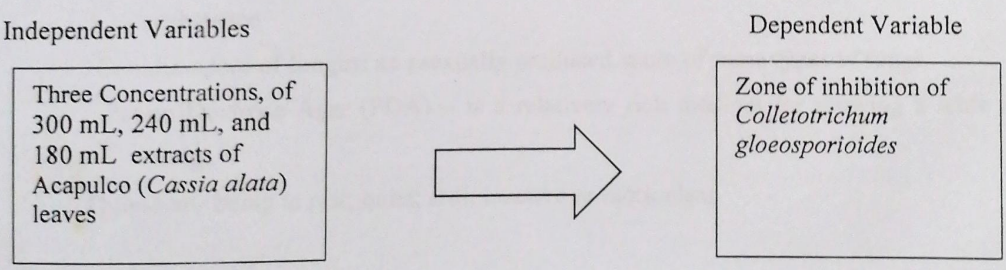
C.B.3. To compare the zone of inhibitions of *Colletotrichum gloeosporioides* after exposure to the three concentrations of 300 mL, 240 mL, and 180 mL after 7 days of incubation

C.B.4. To determine which concentration inhibited the most growth of the fungus, *Colletotrichum gloeosporioides*

D.Hypothesis of the Problem:

There is no significant difference in the zone of inhibition of *Colletotrichum gloeosporioides* when treated with the three concentrations of 300 mL, 240 mL, and 180 mL after 7 days of incubation.

E. Research Paradigm:



Chapter II

REVIEW OF RELATED LITERATURE

This chapter consists of six topics, namely (1) *Colletotrichum gloeosporioides*, (2) *Cassia alata* (Acapulco), (3) Other Plants with Anti fungal Properties, (4) Well diffusion, (5) Zone of Inhibition

A. *Colletotrichum gloeosporioides*

A.1. Description

The genus *Colletotrichum* is a member of the order Melanconiales of the class Coelomycetes (Hawksworth, 1983). It has a wide host range and a worldwide distribution, but is most important in the tropics. Diseases caused by the genus *Colletotrichum* have had a substantial impact on world agricultural production through their capacity to cause economic losses on a number of important cereal, legume, fruit and cash crops (Waller, 1992). The ability of many *Colletotrichum* species to cause latent or quiescent infections places them among the most important of post-harvest pathogens. (http://www.sorghumanthracnose.org)

The fungus produces hyaline, one- celled, ovoid to oblong, slightly curved or dumbbell shaped conidia, 10-15 Âµm in length and 5-7 Âµm in width. Masses of conidia appear pink or salmon colored. The waxy acervuli that are produced in infected tissue are subepidermal, typically with setae and simple, short, erect conidiophores. (http://www.extento.hawaii.edu)

The pathogen initially infects intact, non-wounded, immature, green fruits in the field. Spores germinate and form appressoria on the fruit surface. The fungus, using its appressorium, enzymatically penetrates the cuticle and then remains as sub-cuticular hyphae until the post climacteric stage of fruit growth is attained. At this point, for reasons that are not understood, the fungus resumes growth and causes the characteristic symptoms. Thus, papaya anthracnose has a latent stage in its development that is similar to many other anthracnose diseases of tropical fruits. (http://www.extento.hawaii.edu)

A.2. Life cycle

The life cycle of the *Colletotrichum* species comprises a sexual and an asexual stage. In general terms, the sexual stage accounts for the genetic variability and the asexual stage is responsible for the dispersal of the fungus. Fungal species that reproduce sexually can usually be classified as either self-fertile (homothallic) or self-sterile (heterothallic). (http://www.sorghumanthracnose.org)

A.3. Germination and How it is Spread

Environmental conditions favoring the pathogen are high temperatures, and high humidity. Spores must have free water to germinate; germination is negligible below 97% relative humidity. Spores are only released from acervuli when there is an abundance of moisture. Splashing from rain is a common means of spread. Severity of disease is related to weather and the fungus is relatively inactive in dry weather. Sunlight, low humidity and temperature extremes (below 18Ã»C or greater than 25Ã»C) rapidly inactivate spores. (http://www.extento.hawaii.edu)

A.4. Fruits Infected by *Colletotrichum gloeosporioides*

Colletotrichum is capable of causing disease on virtually all parts of the pepper plant during any stage of plant growth. However, fruit lesions are the most economically important aspect of this disease. Fruit symptoms initially begin as water-soaked lesions that become soft, slightly sunken, and become tan. The lesions can cover most of the fruit surface and multiple lesions occur. The surface of the lesion becomes covered with the wet, gelatinous spores from salmon-colored fungal fruiting bodies (acervuli) with numerous black spines (setae). Concentric rings of the acervuli are common within the fruit spots. In some cases, the lesions are brown, not orange, and then black from the formation of setae and sclerotia (a dark, fungal survival structure). (http://edis.ifas.ufl.edu)

The fungus survives in and on seeds. Anthracnose is introduced into the field on infected transplants or it can survive between seasons in plant debris or on weed hosts. (http://edis.ifas.ufl.edu)

Fruit are infected when spores of the fungus or infested debris is rain splashed onto pepper plants. New spores are produced within the infected tissue and then are dispersed to other fruit. Workers may also move spores with equipment or during handling of infected plants. (http://edis.ifas.ufl.edu)

Infection usually occurs during warm, wet weather. Temperatures around 80Â° F (27Â° C) are optimum temperatures for disease development, although infection occurs at both higher and lower temperatures. Severe losses occur during rainy weather because the spores are washed or splashed to other fruit resulting in more infections. The disease is more likely to develop on mature fruit that is present for a long period on the plant, although it can occur on both immature and mature fruit. (http://edis.ifas.ufl.edu)

Resistance is available in some varieties of chili peppers but not in bell peppers. For bell pepper production, choose cultivars that bear fruit with a shorter ripening period which may allow the fruit to escape infection by the fungus. Wounds in fruit from insects or other means should be reduced to the extent possible because wounds provide entry points for *Colletotrichum* spp. and other pathogens like bacteria that cause soft rot. (http://edis.ifas.ufl.edu)

Mango anthracnose, caused by the fungus *Colletotrichum gloeosporioides*, is the most widespread and serious pre- and post harvest disease of mango. Although infection of of blossom or young fruit can result in failure to produce fruit, the most damaging phase of the disease begins as a quiescent infection, when the fruit is in the preclimacteric phase of development. Growth of the pathogen is resumed after harvest when the fruit starts to ripen and post harvest anthracnose develops.

A.5. Different Forms of Controlling its Spread and Effects

Hot water dips at 48Ã»C for 20 min is an effective treatment for reducing anthracnose incidence. Although hot water dips do not completely eliminate anthracnose the reduction in disease is economically significant. (http://www.extento.hawaii.edu)

Orchard sprays applied at 14 - 28 day intervals, depending on rainfall, with an appropriate protective fungicide is commonly recommended. Postharvest fungicides, applied as a spray or dip, with a food-grade wax have also shown to be effective in reducing anthracnose. This is a common practice especially for fruits shipped to overseas markets. (http://www.extento.hawaii.edu)

Control of the disease caused by *Colletotrichum gloeosporioides* is through integrated management techniques. The disease should not be introduced on infected plants. Only seeds that are pathogen-free should be planted. Transplants should be kept clean by controlling weeds and solanaceous volunteers around the transplant houses. The field should have good drainage and be free from infected plant debris. If disease was previously present, crops should be rotated away from solanaceous plants for at least 2 years. Sanitation practices in the field include control of weeds and volunteer peppers plants. (http://edis.ifas.ufl.edu)

For late-maturing peppers, when disease is present, apply a labeled fungicide several weeks before harvest. The disease can be controlled under normal weather conditions with a reasonable spray program. At the end of the season, remove infected plant debris from the field or deep plow to completely cover crop diseases. (http://edis.ifas.ufl.edu)

Blossom sprays applied at fortnightly intervals, with iprodione (50g a.i./100L), chinomethionate (6.25g a.i./100L), prochloraz manganese chloride (12.5g a.i./100L), triadimenol (5g a.i./100L), copper oxychloride (225g a.i./100L), mancozeb (160g a.i./100L), flusilazol (2g a.i./100L), polysulphide sulphur (320a.i./100L) and pyrazophos (11.5g a.i./100L) were evaluated over two seasons (1989-1991) for the simultaneous control of powdery mildew (*Oidium mangiferae*), blossom blight (*Nattrassia mangiferae*) and blossom spot (*Alternaria/Colletotrichum* complex) of mango. Blossom spays with the systemic fungicides flusilazol or pyrazophos resulted in significant disease control, and consistently higher fruit set and yield above the controls. (http://www.up.ac.za)

A.6. Economic Importance on Agricultural Crops

Colletotrichum diseases have been reported on a wide range of hosts. However, the most economically important of these are species attacking cash crops such as cotton, coffee, beans and tropical fruit, such as bananas, mangoes and papayas (Waller, 1992). Losses caused by *Colletotrichum* species mostly occur as a result of the direct reduction of quality and or quantity of the harvested yield. This is most noticeable and severe when *Colletotrichum* directly attacks the harvested portion of the crop, often the fruit. Wider economic consequences are also linked with other factors including the poor return on investment, and the cost of chemical control. Many export crops play a crucial role in the economy of small, developing countries, and disease losses can have a significant impact at the macro-economic level (Waller, 1988). At the micro-economic level, reductions in yield losses have a significant effect on the lives of people, many of whom are subsistence or small-holder farmers, or depend on local markets for food. (http://www.sorghumanthracnose.org)

B. *Cassia alata* (Acapulco)

B.1. Description

Acapulco is an erect tropical, annual herb with leathery, dark green compounded leaves on stout branches. It grows up to 6' tall. This perennial shrub has erect waxy yellow spikes that resemble fat candles before the individual blossoms open. An axis of golden yellow flowers produces 4-winged pods containing 50-60 flattened, triangular seeds. Flowers enclosed by yellow-orange bracts that are later shed. The large leaves are bilateral - symetrical opposed, fold together at night and have orange rachis. The fruit is a pod, while the seeds are small and square. It was introduced to other tropical areas from the Americas and is now widely considered a weed. (http://www.philippineherbs.com, http://www.2.com, http://www.3.com, http://www.4.com)

B.2. Uses

The leaves of *Cassia alata* contain chrysophanic acid (chrysophanol) and are reported to be sudorific, diuretic and tincture from leaves reported to be purgative. The leaves are commonly used for ringworm and other skin diseases. The decoction of leaves and flowers are also used to treat bronchitis and asthma. Crushed leaves and juice extract are used to treat ringworm, scabies, eczema, tinea infections, insect bites, herpes. Because of its anti-fungal properties, it is a common ingredient in soaps, shampoos, and lotions here in the Philippines. The Philippine Council for Health Research and Development (PCHRD) has helped develop the technology for acapulco lotion.

Other chemicals contained in the plant includes saponin which acts as a laxative and expels intestinal parasites. In Africa, the boiled leaves are used to treat high-blood pressure. In South America,

besides skin diseases, it is also used to treat a wide range of ailments from stomach problems, fever, asthma to snake bite and venereal diseases like syphilis, gonorrhea. (www.philippineherbs.com, www.2.com, www.4.com)

B.3 Cultivation

Cassia alata grows best with full sun or with only light shade. The plant prefers moist well drained soil and it is tolerant to drought. It can also be kept as a container plant. In planting *Cassia alata*, start indoors and then transplant. It should be planted after last frost or better. (www.3.com)

C. Other Plants with Antifungal Properties

C.1. *Melaleuca alternifolia*

Tea tree oil (*Melaleuca alternifolia*) is a multi-purpose herb that traces its roots to the Aboriginal people of Australia. For thousands of years, they used the leaves as an antiseptic and antifungal by crushing the leaves and making a mudpack. When crushed and distilled, the leaves of this plant yield a 100% natural oil which is an antiseptic, a fungicide, and a mild solvent. (http://www.sunspirit.com)

Tea tree oil's properties are contained in the oils of its leaves. The oil is steam distilled from the leaves and then tested for chemical properties, which can number between 50 and 100. This may explain tea tree oil's many beneficial uses. The main active components are terpinen-4-ol, 1, 8-cineole, gamma-terpinene, p-cymene and other turpenes. Terpinen-4-ol (typically 30-40%) is responsible for tea tree oil's antibacterial and antifungal properties. Its aroma is one of a healthy pleasant disinfectant. (http://www.sunspirit.com)

With its antifungal and antibacterial properties, tea tree oil works well as a body cleanser or shampoo. Not only does tea tree fight fungal infections, but also, as a natural solvent, it helps dissolve toxins that cause itching. Once itching from rashes, dry scalp, dandruff, sunburn, or fungal infections stop, your skin heals faster. Also, without itching and scratching, you avoid even more serious infections. (http://findarticles.com)

Tea tree oil has pain-numbing properties and can be used topically for sprains, arthritis, bunions, bursitis, eczema, gout, carpal tunnel syndrome, and hemorrhoids. It is best to use products containing essential tea tree oil, since the pure essential oil would be irritating to sensitive areas. (http://findarticles.com)

A study at the Flinders University of Adelaide is currently researching tea tree oil's affects on various inflammations in the body. The goal is to discover if the essential oil reduces the inflammation besides killing the microorganisms causing it. (http://findarticles.com)

For relief from pain caused by the various arthritic afflictions (rheumatoid arthritis , osteoarthritis, etc.), combine 18 drops of tea tree oil with 1/8 cup of almond oil. Put in a dark bottle and shake before applying it topically two to four times a day as massage oil. It dan also be used to massage the wrists for carpal tunnel syndrome. Or add a dozen drops of tea tree oil to your bath water and soak in it. (http://findarticles.com)

Tea tree oil's antifungual properties also are well-documented. A double-blind study published in the Journal of Family Practice (June 1994) found that pure tea tree oil relieved nail fungus as effectively as 1 percent clotrimazole, a topical antifungal drug. And in 1985, researchers at the University of Paris studied 28 women who used tea tree oil suppositories to combat Candida albicans, the common yeast infection. After one month, 21 women showed a complete recovery. Dilute a few drops of tea tree oil in a spoonful of water, put it on a tampon and leave inserted for 24 hours. (http://findarticles.com)

C.2. *Azadiracta indica*

Affectionately called "the village pharmacy," India's neem tree is practically a first-aid kit in itself. Packed with the antibacterial, antifungal, antiviral, antihistamine, antiseptic, spermicidal and immune-system stimulating components nimbin and nimbidin, neem is said to do everything from repelling insects to preventing pregnancy. "It's a great family first-aid herb," says Karta Purkh Singh Khalsa, a Seattle-based herbalist and co-author of Herbal Defense (Warner Books, 1997). (http://findarticles.com)

Oil extracted from neem seeds covers plant leaves like a raincoat, stopping fungi that cause diseases such as powdery mildew and rust from infecting plants. Fungal spores are spread by wind and splashing raindrops. "If the spores can't adhere to a leaf, germinate, and penetrate the leaf cells, they can't cause disease," says Jim Locke, an ARS research plant pathologist. (http://findarticles.com)

Almost all parts of the versatile plant contain extractable compounds that have been used for centuries in India in personal hygiene products like soap and toothpaste. Seed extract has been used to treat skin diseases, sores, and rheumatism. (http://findarticles.com)

Locke says that in numerous tests, a spray of 1-percent neem oil in water "stopped 95 to 100 percent of the powdery mildew on hydrangeas, lilacs, and phlox." Single spray application was sufficient to protect these ornamentals from infection. Repeated applications at 7- to 14-day intervals as the plants grew provided disease protection without any plant damage (http://findarticles.com)

On plants where mildew had begun to develop, "it was arrested," he says, "providing control comparable to each of three chemical fungicides." Powdery mildew, which also attacks crepe myrtles and ros s, causes leaves to turn white. Preliminary results indicate the oil will arrest and control the fungus that plagues these popular ornamentals, especially in humid areas. Locke says the oil is the first botanical

product to exhibit fungicidal properties. He has been field-testing it for the past 4 years on several greenhouse and nursery crops. One study involves numerous laboratory tests of roses by Locke's group in the Floral and Nursery Plants Research Unit at the agency's U.S. National Arboretum, Washington, D.C. He says the oil "seems to delay infection by black spot--the number-one disease of roses. As a result, rose bushes lose fewer leaves, compared to untreated, diseased plants." (http://findarticles.com)

Locke says this research, begun in cooperation with former ARS entomologist Hiram Larew, also demonstrated that neem oil can reduce damage caused by various pests, including spider mites. "In preliminary tests, a 2-percent spray of neem seed oil applied directly to spider mite eggs resulted in 87-percent mortality," he says. Research at USDA on plant-derived natural pesticides, such as nicotine, dates back to the 1920's. Beginning in 1975, extraction products from neem seeds were evaluated for their insect-killing properties. (http://findarticles.com)

Neem oil is found primarily in topical health and beauty products, where its strong scent (akin to raw garlic or burnt coffee) is often masked by more pleasant oils. Although few studies have been done on neem oil, 4,500 years of continued use bear out its efficacy: Added to toothpaste and mouthwash, it prevents cavities and gingivitis; in creams (containing at least 25 percent neem oil), it combats vaginal infections and sexually transmitted diseases; in soaps and shampoos, it kills lice, ringworm and scabies; mixed with equal parts vegetable oil and water, it makes a healing soak for athlete's foot; undiluted, it repels fleas, ticks, mosquitoes and flies. And test tube and human studies done at India's Defense Institute of Physiology and Allied Science found that neem oil mixed with Indian soap nut extract and quinine was nearly 100 percent effective as a spermicide. (http://findarticles.com)

C.3. *Origanum vulgare*

Studies on the biological activity of oregano show a wide range of antibacterial, antifungicidal, antiviral and antioxidant properties for the essential oil and extracts. In recent years, oregano has moved from fame as a culinary herb into increasing popularity as a dietary supplement, sparked by interest in the essential oil through aromatherapy.

The oil of the oregano plant has received some recent attention as a natural means to combat bacterial and fungal infections. Researchers at the University of Tennessee compared the power of various spices in fighting off common food borne microbes, including E. coli and Listeria. The results were startling: Oil of oregano seemed to completely inhibit the growth of harmful organisms. Cass Ingram, M.D., author of The Cure is in the Cupboard, says that oil of oregano can treat conditions ranging from acne and earaches to varicose veins. Ingram even suggests taking a few drops before eating in a restaurant to fight off any bacteria that might lurk in the salad bar.

A soon-to-be published study by Dr. Preuss and colleagues looked at the potential of oregano oil in *Candida albicans* (candida), and found that oregano demonstrated antifungal powers in these test-tube and experimental studies, against both systemic and skin infections. (http://findarticles.com)

Like many essential oils, oil of oregano has significant antibacterial and antifungal abilities. However, just because these effects can be achieved in a laboratory does not mean that these effects translate to humans when ingested. With the recent anthrax scare, some marketers have suggested -- even promoted -- the antibacterial activity of oregano oil in anthrax. But there is no credible evidence to suggest that oregano oil could be directly useful for treatment of anthrax. The Federal Trade Commission has done consumers a service by warning such purveyors to cease such questionable, fraudulent claims. (http://findarticles.com)

C.4. *Raphanus sativus* (Radish)

The radish is a hairy annual herb. It has no stem or branches. It has only one root which is round, cylindrical or tapering, white or red, slightly coarse, fleshy, succulent and 3 to 7 cm thick, 10 to 20 cm long. It has a pungent flavor. It is hardy to zone 0 and is not frost tender. They are tender when young but become tough and woody as they mature. It is in flower from June to August, and the seeds ripen from July to September. The flowers are hermaphrodite (has both male and female organs) and are pollinated by bees and flies. There are many varieties of radish, differing size and color. The red and white colored are most familiar. It stimulates appetite and promotes a healthy bloodstream. (http://www.ibiblio.org, http://www.indiangyan.com)

The plant prefers light (sandy), medium (loamy) and heavy (clay) soils. The plant prefers neutral and basic (alkaline) soils. It can grow in semi-shade (light woodland) or no shade. It requires moist soil. (http://www.ibiblio.org)

Its medicinal uses are anthelmintic; antibacterial; antifungal; antiscorbutic; antispasmodic; astringent; cancer; carminative; cholagogue; digestive; diuretic; expectorant; laxative; poultice; stomachic. Radishes have long been grown as a food crop, but they also have various medicinal actions. Radishes are also an excellent food remedy for stone, gravel and scorbutic conditions. The roots stimulate the appetite and digestion, having a tonic and laxative effect upon the intestines and indirectly stimulating the flow of bile. The root is antiscorbutic, antispasmodic, and astringent, digestive and diuretic. It is crushed and used as a poultice for burns, bruises and smelly feet. The root is best harvested before the plant flowers. Its use is not recommended if the stomach or intestines are inflamed. Consuming radish generally results in improved digestion, but some people are sensitive to its acridity and robust action. The leaves, seeds and old roots are used in the treatment of asthma and other chest complaints. Green leaves of radish are beneficial in treatment of jaundice. The juice of the fresh leaves is diuretic and

laxative. The seed is carminative, diuretic, expectorant, laxative and stomachic. It is taken internally in the treatment of indigestion, abdominal bloating, wind, acid regurgitation, diarrhea and bronchitis.

The plant contains raphanin, which is antibacterial and antifungal. It inhibits the growth of Staphylococcus aureus, E. coli, streptococci, Pneumococci etc. Raphaniń (sulforaphen) is a compound found in radish seeds and leaves. The plant also shows anti-tumour activity. (http://www.ibiblio.org, http://www.indiangyan.com).

Radishes are a popular choice for personal cultivation, as they are fairly easy to grow. It is a rapidly-maturing crop, with many varieties able to reach maturity within 30 days.

Radishes grow best in full sun and moist, fertile, acidic to neutral soil. They are in season April, May, June, July, August, September, and part of October. Seeds will germinate if planted at a depth of 1 cm. (http://en.wikipedia.org)

Raphanus sativus is a very easily cultivated fast-growing plant which prefers a rich light soil with ample moisture. They dislike very heavy or acid soils. Plants are susceptible to drought and require irrigation during dry spells in the summer or the root quality will rapidly deteriorate and the plant will go to seed. Radishes are widely cultivated for their edible roots. There are many named varieties that are able to supply edible roots all year round. Over the centuries a number of distinct groups have evolved through cultivation, these have been classified by the botanists as follows. Radishes are a good companion plant for lettuces, nasturtiums, peas and chervil, tomatoes and cucumbers. They are said to repel cucumber beetles if planted near cucumber plants and they also repel the vine borers which attack squashes, marrows and courgettes. They grow badly with hyssop and with grape vines. (http://www.ibiblio.org)

D. Well Diffusion

In agar well diffusion assay, wells are made on agar plates using a sterile borer. The antifungal activity is evidenced by the presence of a zone of inhibition surrounding the well. Zone sizes are measured in millimeters. (http://www.bioline.org)

E. Zone of Inhibition

Antibiotic discs are often used to determine if a particular bacterium is susceptible to a type of antibiotic. The bacteria are grown on a dish and discs saturated with different antibiotics are placed on top of the growing bacteria. If the antibiotic works successfully, a clear ring will appear around the disc in 24/48 hours. The ring is called the zone of inhibition. It is measured in mm to see how wide it is. The larger this zone of inhibition, the more effective that antibiotic is against that particular type of bacteria. (http://www.hhmi.org)

Chapter III
METHODOLOGY

A. Materials

Petri plates
Test tubes
Test tube rack
Wash bottle
Stirring rod
Beakers
Graduated cylinders
Inoculating loop
Potato Dextrose Agar
Incubator
Autoclave
Alcohol lamp
Pruning scissors
Distilled water
Sealable plastic bags
Camera
Alcohol
Weighing scale
Hot plate
Mortar
Pestle
Forceps
Lysol
Matches
Dishwashing liquid
Face mask
Cork borer

B. Methods

B.1. Collection of Specimen

B.1.1. Collection of Test Organisms

The pure cultures of *Colletotrichum gloeosporioides* were ordered from Philippine National Collective of Microorganisms- UP Los Baños. The cultures were refrigerated until use.

B.1.2. Collection of Leaf Specimens

The leaves of medicinal plant Acapulco (*Cassia alata*) were gathered from Brgy. Bito-on, Jaro, Iloilo City. These leaves were washed with distilled water and were ovendried at 50 degrees Celsius overnight.

B.2. Preparation of the Leaf Extracts and the Concentrations

Cassia alata leaves were cut into small pieces. The cut leaves were measured using the electronic balance. The leaves weighed 31.8 grams and were divided into three equal parts. 10.6 grams of leaves were transferred into the first glass jar and was added with 300 milliliters of methanol. 10.6 grams of leaves were transferred into the second jar and was added with 240 milliliters of methanol. The remaining 10.6 grams of leaves were transferred to the third jar and was added with 180 milliliters of methanol. These jars were covered tightly and were left for two weeks for the extraction.

B.3. Sterilization of Equipment

The Petri plates, beakers, test tubes and graduated cylinder were washed and placed in the oven to dry. The Petri plates were covered with paper. These, together with the beakers, test tubes, stirring rod and graduated cylinders, were autoclaved at 120 degree Celsius at 15 psi for 15 minutes. Any equipment that was not completely dry was placed in the oven again.

B.4. Preparation of Culture Media

The media used was the Potato Dextrose Agar (PDA). 15.6 grams of PDA was mixed with 400 milliliters of distilled water. The mixture was cooked with frequent agitation and was boiled for 1 minute to completely dissolve the powder. The cooked media was autoclaved at 121 psi for 15 minutes.

20 milliliters of the media was measured using a sterilized graduated cylinder and was poured to the Petri plate. The same amount was poured to the remaining plates. 20

plates were poured with agar and was left to solidify for an hour. The media plates were wrapped in paper and were refrigerated until use.

B.5. Preparation of the Wells

4 millimeter holes were aseptically punched in the culture media plates using a cork borer. The cork borer was autoclaved for 15 minutes. Each plate was divided into six equal parts using a protractor and marker. The flow hood was sterilized by heating a solution of one-half teaspoon of potassium permanganate dissolved in 20 milliliters of distilled water. After all the solution evaporated, the inside of the flow hood was wiped with ethanol and sprayed with Lysol.

The Petri plate containing the culture medium was heated around the edge. A hole was punched in the center of each part. 6 holes were punched in each plate. The holes were equal in distance from each other and from the approximated center of the Petri plate. After every 3 plates were punched with holes, the cork borer was sterilized to ensure minimal contamination. 15 plates were punched using the cork borer. The plates were refrigerated again until use.

B.6. Preparation of Subculture of *Colletotrichum gloeosporioides*

The culture of *C. gloeosporioides* obtained from UP Los Baños was subcultured. The flow hood was sterilized by heating a solution of one-half teaspoon of potassium permanganate dissolved in 20 milliliters of distilled water. After all the solution evaporated, the inside of the flow hood was wiped with ethanol and sprayed with Lysol.

The sterilized inoculating loop was heated using an alcohol lamp until the loop turned red. The Petri plate containing the culture medium was also heated around the edge. The heated loop was left to cool down for a few seconds before scraping from the original culture. *C. gloeosporioides* was scraped off from the original culture using the loop and was deposited at the center of the medium. The Petri plate was heated again around the edge to prevent contamination. The same procedure was followed for the other plates. 5 plates were used to subculture *C. gloeosporioides.* The cultures were left inside the flow hood for 7 days to ensure maximum growth.

B.7. Transfer of the Extracts and of the Pathogen

The underside of the plates was labeled using price tags and markers. The first hole was labeled as water for the negative control, the second as 300 for the 300 mL

concentration, the third as + for the positive control Armor, the fourth as 240 for the 240 mL concentration, the fifth as CS for the control solvent methanol and the sixth as 180 for the 180 mL concentration.

The flow hood was sterilized by heating a solution of one-half teaspoon of potassium permanganate dissolved in 20 milliliters of distilled water. After all the solution evaporated, the inside of the flow hood was wiped with ethanol and sprayed with Lysol.

The Petri plate containing the agar with wells was heated around the edge. Using a syringe without a needle, the well labeled with 300 was filled with the 300 mL concentration. This was done as fast as possible to minimize the medium's exposure. The same was done with the other wells labeled 300. After all the wells labeled with 300 were filled, the syringe was put in a beaker.

Using another syringe without a needle, the well labeled with 240 was filled with the 240 mL concentration. This was done as fast as possible to minimize the medium's exposure. The same was done with the other wells labeled 240. After all the wells labeled with 240 were filled, the syringe was put in a beaker.

This procedure was repeated for the 180 mL concentration extracts as well as for the positive control, negative control and the control solvent, each time using a different syringe without a needle.

After all the plates' wells were filled with their corresponding extracts and solutions, the pathogen was transferred in each plate by using the fungal block method. A scalpel was autoclaved for 15 minutes and was again heated using the alcohol lamp. The scalpel was left to cool for a few seconds. Using the scalpel, a square block about 1 cm in width and length was cut from a plate with the subcultured *C. gloeosporioides* and was transferred into the center of the plates with wells. This procedure was repeated until all the 15 plates had a block of pathogen. This was done as fast and as accurately as possible to minimize exposure. These plates were left inside the flow hood for 7 days to ensure maximum growth.

B.8. Measurement of the Zone of Inhibition

The zone of inhibition will be measured using a ruler. Zone diameters will be read at the point where growth decreased abruptly. The results will be recorded tabulated.

B.9. Disposal of Fungi

The plates were sprayed with Lysol and were autoclaved to kill all the microorganisms. The agar from the plates was scraped off and thrown properly to ensure that none of the pathogen spreads in the environment. The plates were washed and were oven dried. All materials and equipment were returned to the SRA.

Chapter IV

RESULTS AND DISCUSSION

This study aimed to determine the antifungal reaction of *Colletotrichum gloeosporioides* to Acapulco (*Cassia alata*) leaf extracts.

Specifically, it aimed to determine and compare the zones of inhibition of *Colletotrichum gloeosporioides* after exposure to the three concentrations of 300 mL, 240 mL, and 180 mL after 7 days of incubation. It further aimed to determine which concentration inhibited the most growth of the fungus, *Colletotrichum gloeosporioides*.

It was hypothesized that there is no significant difference in the zones of inhibition of *Colletotrichum gloeosporioides* when treated with the three concentrations of 300 mL, 240 mL, and 180 mL after 7 days of incubation.

A. Results:

During the subculturation of the pathogen *Colletotrichum gloeosporioides*, the fungi did not grow well. Instead, different species of unknown fungi were observed to grow in the plates. Also, the plates punched with holes were observed to have growths of unknown fungi even before the *C. gloeosporioides* was transferred in them.

On other trials the fungi *C. gloeosporioides* grew when subcultured. Only one plate was observed to have any foreign growth. When the blocks were transferred into the plates with wells filled with the extracts, the fungi grew. Only two of the plates have any foreign growth, though very minimal. It was observed that in all the plates, the extracts did not inhibit the growth of the pathogen. Also, *C. gloeosporioides* grew all over the control solvent methanol but was inhibited by the positive control Armor. Around the wells filled with the negative control water, foreign growth was observed. The fungi *C. gloeosporioides* did not grow around these wells.

On another trial the fungi *C. gloeosporioides* grew when subcultured. Two of the plates were observed to have foreign growth. When the blocks were transferred into the plates with wells filled with the extracts, *C. gloeosporioides* grew. It was observed that all of the plates have foreign growth in them.

B. Discussion:

In the first try, the fungi did not grow well because there was a lot of foreign growth in the plates. The flow hood was not properly cleaned and sterilized, and thus was the apparent cause of the foreign growths.

A cork borer was used in punching the holes and an aspirator was used to suck the agar from the holes. The plates were exposed openly inside the unsterilized flow hood for quite sometime until all the holes were punched. This had caused the unwanted growths even though the plates have been refrigerated again after the wells were punched. Also, the aspirator cannot be autoclaved nor heated so the air inside it may contain contaminants. This might have contributed to foreign fungi's growth.

In the second try, the flow hood was cleaned and sterilized by spraying Lysol. The aspirator was not used to suck the agar. Only the cork borer was used to make the holes. After every 3 plates were punched with holes, the cork borer was sterilized again. Also, in transferring the extracts to the wells, sterile syringes without needles were used. The plates were exposed for a very short period of time since the transfer of extracts was done as fast as possible.

The transfer of fungal blocks was also done as fast as possible to minimize contamination. The scalpel used to cut the blocks was autoclaved before use. After two days, it was observed that *C. gloeosporioides* was growing. Also, the wells around the negative control water were observed to have shiny growths which were very different from the appearance of *C. gloeosporioides*. The distilled water used as the negative control was already opened for quite sometime and may contain yeast thus, explaining the growths around the wells filled with water.

After 7 days of incubation, the fungi *C. gloeosporioides* almost covered the surface of the media except around the wells of the positive control Armor and the wells of distilled water.

In the third try, the flowhood was sterilized by heating a solution of potassium permanganate dissolved in water. The whole inside of the flowhood was wiped with ethanol and was then sprayed with Lysol.

The same procedure as in the second try was done. After two days, the growth of *C. gloeosporioides* was minimal but a lot of foreign growth was observed in every plate.

Chapter V

CONCLUSION AND RECOMMENDATIONS

- Summary

This study aimed to determine the antifungal reaction of *Colletotrichum gloeosporioides* to Acapulco (*Cassia alata*) leaf extracts.

Specifically, it aimed:

- To determine and compare the zones of inhibition of *Colletotrichum gloeosporioides* after exposure to the three concentrations of 300 mL, 240 mL, and 180 mL after 7 days of incubation.
- To determine which concentration inhibited the most growth of the fungus, *Colletotrichum gloeosporioides.*

Summary of results:

Based on the attempts, it was observed that the extracts did not inhibit the growth of *Colletotrichum gloeosporioides* although the positive control, Armor, inhibited its growth.

However no substantive result could be achieved because of the presence of contaminants.

- Conclusion

- Recommendations

It is recommended that maximum sterility should be ensured in order to prevent contamination. Since not one concentration showed a significant difference in the growth of *Colletotrichum gloeosporioides,* it is recommended that different concentrations may be used in the next experiments to determine whether other concentrations of the methanol extracts of Acapulco (*Cassia alata)* can inhibit the growth of the fungus. Aside from changing the concentrations, the solvent used can also be changed because other chemicals can be substituted to dissolve the leaf extracts of Acapulco (*Cassia alata*).

LITERATURE CITED

No author. (n.d.). Anthracnose. Available at: http://www.umassturf.org/publications/fact_sheets/diseases/control_anthracnose.pdf via the INTERNET. Accessed on 2006 Dec 16.

No author. 2003. Anthracnose. Available at: http://www.ipm.ucdavis.edu/PMG/PESTNOTES/ph7420.html via the INTERNET. Accessed on 2006 Nov 9.

No author. (n.d.). Biotrophic plant pathogens. Available at: http://helios.bto.ed.ac.uk/biotroph.htm via the INTERNET. Accessed on 2006 Sept 13.

No author. (n.d.). Botrytis. Available at: http://plantclinic.cornell.edu/Factsheet/botrytis/botrytis-blight.htm via the INTERNET. Accessed on 2006 Sept 13.

No author. (n.d.). Colletotrichum. Available at: http://www.plant.uga.edu/Extension/Fungi/Colletotrichum.html via the INTERNET. Accessed on 2006 Sept 13.

No author. (n.d.). Monilinia. Available at: http://biologi.uio.no/bot/ascomycetes/Taxa/Monilinia.html via the INTERNET. Accessed on 2006 Sept 13.

No author. (n.d.). Potato Dextrose Agar. Available at: http://service.merck.de/microbiology/tedisdata/prods/4985-1_10130_0500.html via the INTERNET. Accessed on 2006 Oct 18.

No author. (n.d.). Traditional medicinal knowledge about *Haplotriphs ramakrishnae* K.(Thysanopthera: Phloeothripidae) feeding on Guktaudi (*Chrysanthemum indicum*) in Chhattisgarh, India. Available at: http://botanical.com/site/column_poudhia/articles/_1582.html via the INTERNET. Accessed on 2006 Sept 10.

Bajwa R., Khalid A., Cheema T.S. 2003. Antifungal Activity of Allelopathic Plant Extracts III: Growth Response of Some Pathogenic Fungi to Aqueous Extract of *Parthenium hysterophorus*. Pakistan Journal of Plant Pathology [serial online]; 2(3):145-156. Available at: http://scholar.google.com/scholar?hl=en&lr=&q=cache:7IkDIgN_n0IJ:ansinet.org/fulltext/ppj/ppj23145-156.pdf+plants+that+have+antifungal+activity+philippines via the INTERNET. Accessed on 2006 Nov 7.

Becker H. 1994. Neem Oil Locks Out Spores. Agricultural Research [serial online]. Available at: http://findarticles.com/p/articles/mi_m3741/is_n6_v42/ai_16084668. Accessed on 2007 Jan 10.

Beever R.E., Plummer K.M., Wurms K.V. 2005. Novel Approaches to Controlling Fruit Pathogens. New Zealand Plant Protection [serial online]; 58:63-73. Available at: http://www.nzpps.org via the INTERNET. Accessed on 2006 Sept 24.

Benyahia H., Jrifi A., Smaili C., Afellah M., Timmer L.W. 2003. First report of *Colletotrichum gloeosporioides* Causing Withertip on Twigs and Tear Stain on Fruit of Citrus in Morocco. Available at: http://www.bspp.org.uk/ndr/july2003/2003-25.asp via the INTERNET. Accessed on 2007 Jan 19.

Canton E., Pernan J., Gobernado M., Viudes A., Espinel-Ingroff A. 2004. Synergistic Activities of of Fluconazole and Terbinafine against Four Candida Species Determined by Checkerboard, Time-kill, and Etest Methods. Available at: http://aac.asm.org/egi/content/full/49/4/1593.

Case C.L. (n.d.). Discover A New Antibiotic: Tests for Antimicrobics. Available at: http://www/smccd.net/accounts/case/antibiotics4.html#anchor277665 via the INTERNET. Accessed on 2007 Mar 20.

Case C.L. Soil Productivity-Plate Count Method. Retrieved from: http://www.smccd.net/accounts/case/envmic/plate_count.html on Sept. 27, 2006.

Chen C., Dickman M.B. 2005. Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*. n.d. Accessed on 2006 Set 24.

Dickman M.B. 1993. Colletotrichum gloeosporioides. Available at: http://www.extento.hawaii.edu/Kbase/crp/type/c_gloeo.htm via the INTERNET. Accessed on 2007 Jan 19.

Estrada A.B., Dodd J.C., Jeffries P. 2000. Effect of humidity and temperatures on conidial germination and appresorium development of two Philippine isolates of the mango anthracnose pathogen *Colletotrichum gloeosporioides*. Plant Pathology [serial online]; 49-608-618. Available at: http://blackwell-synergy.com/links/doi/10.1046/j.1365-3059.2000.00492_x/full/ via the INTERNET. Accessed on 2006 Oct 28.

Falahati M., Tabrizib N.O., Jahaniani F. 2005. Anti Dermatophyte Activities of Eucalyptus camaldulensis in Comparison with Griseofulvin. Iranian Journal of Pharmacology & Therapeutics; 4(2):80-83. Available at: http://www.bioline.org.br/request?pt05018 via the INTERNET. Accessed on 2007 Mar 20.

Jensen K. (n.d.). Colletotrichum gloeoporioides (Penz.) Penz. & Sacc. F. sp. Hypericum. Fungal disease. Available at: http://sci.agr.ca/lethbridge/weedbio/agents/acolglo_e.htm via the INTERNET. Accessed on 2006 Nov 16.

Klepser M., Ernst E., Lewis R., Ernst M., Pfaller M. 1998. Influence of Test Conditions on Antifungal Time-Kill Curve Results: Proposal for Standardized Methods. Available at: http://www/pubmedcentral.nih.gov/botrender.fegi?blobtype=html&artid=105779 via the INTERNET. Accessed on 2006 Nov 12.

Ma Y.Q., Cheng J.M., Inanaga S., Shui J.F. Introduction and Inhibition of *Striga hermonthical* (Del.) Benth. Germination by Extracts of Traditional Chinese Medicinal Herbs. Available at: http://lagron.scijournals.org/cgi/content/full/96/5/1349?ck=nck via the INTERNET. Accessed on 2006 Sept 10.

Pfaller M.A., Sheehan D.J., Rex J.H. 2004 April. Determination of Fungicidal Against Yeasts and Molds: Lessons learned from Bactericidal Testing and the need for Standardization. Clinical Microbiology Reviews [serial online]; 17(2):268-280. Available at: http://cmr.asm.org/cgi/content/full/17/2/268. via the INTERNET. Accessed 2006 Nov. 16.

Roberts P.D., Pernezny K.L., Kucharek T.A. 2001. Anthracnose Caused by Colletotrichum sp. on Pepper. Available at: http://edis.ifas.ufl.edu/PP104 via the INTERNET. Accessed on 2006 Nov 10.

Schumann G. (n.d.). Why is it so Difficult to Control Anthracnose?. Available at: http://66.102.7.104/search?q=cache:j4dwEWHmHd0J:www.umassturf.org/publications/fact_sheets/diseases/control_anthracnose.pdf+how+to+control+anthrcanose&hl=en&gl=ph&ct=clnk&cd=3 via the INTERNET. Accessed on 2006 Nov 27.

Singh A.P. Novel Herbal Research Molecules. Available at: http://www.selfgrowth.com/articles/Singh.html via the INTERNET. Accessed on 2006 Sept 10.

TeBeest D., Cisar C. (n.d.). Sexual Cycle and Potential for Gene Flow in Fungal Biological Control Agents. Available at: http://www.isb.vt.edu/brarg/brasym94/tebeest.htm via the INTERNET. Accessed on 2006 Oct 5.

Yourman L.F., Jeffress S.N. 1999. Resistance to Benzimidazoleand Dicarboximide Fungicides in Greenhouse Isolates of *Botrytis cinerea*. The American Phytopathological Society [serial online]; 83:569-575. Available at: the internet via the INTERNET. Accessed on 2006 Aug 25.

APPENDIX A

PLATES

Plate 1. Two foreign fungi grew on this plate. Positive control Armor inhibited *C. gloeosporioides* growth.

Plate 2. *C. gloeosporioides* during its fourth day of growth.

Plate 3. After 5 days, the growth of *C. gloeosporioides* was inhibited by positive control Armor.