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IMPACT OF PLANT OILS AS ANTIFUNGAL ACTIVITY AGAINST FUNGAL PATHOGENS OF CINNAMOMUM ZEYLANICUM (CINNAMON)

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ABSTRACT

Eleven fungal species were isolated from cinnamon plant, and two fungal genera could not be identified because they failed to sponulate in pure cultures. The identified species of fungi belonged to the genera Curvularia, Helminthosporium, Pestalotiopsis, Aspergillus, Rhizopus, Cladosporium, Nigrospora and Trichoderma.The effects of plant oils and synthetic fungicides were studied in a series of experiments against fungal pathogens of Cinnamon. The present results indicated a range of plant oils over the concentration range of 0.1-3% is capable of inhibiting fungal spore germination on Cinnamon. Several oils were shown to be more efficient than the synthetic fungicide Amister at field concentrations. The oils of cinnamon bark and clove provided the greatest inhibition of spore germination of most of the fungal provided the greeted inhibition of spore germination of most of the fungal species tested. Cinnamon oils completely inhibited spore germination of most fungi over the whole range of concentrations tested, and especially at low concentration. Also, the results revealed tea-tree oil (TO) significantly inhibited germination of all of the fungi isolated from cinnamon at concentrations of 0.1 or 0.2% with the exception of Curvularia inaequalis which responded only at the highest concentration (3%). lemon grass oil (LgO) provided that complete inhibition of Pestalotiopsis (C21) spore germination occurred at all concentrations tested. Meanwhile, complete inhibition of germination on Curvularia sp. (C30) occurred at 1 or 3 concentration. I can concluded that the oil lemon grass greatest suppress the germination of fungi isolated from Cinnamon.

Keywords: Plant oils, Synthetic fungicides, *Curvularia*, *Helminthosporium*, *Pestalotiop*sis, *Aspergillus*, *Rhizopus*, *Cladosporium*, *Nigrospora* and *Trichoderma*.

1. INTRODUCTION

Plant diseases is an om-going limiting factor in crop production.Diseases of crops lead to yield losses and are of increasing importance as world population increases [1]. Plant diseases are of paramount significance to humans because they damage plants and plant products on which humans depend for food, clothing and fumiture [2]. Fungi are the most important common cause of plant disease [1], since they are the most widespread and destructive parasites of plants [3].

Pathogenic fungi lead to changes in developmental stages In fruit and vegetables and cause problems related to quality and nutritional value [4]. In order to control plant diseases around the world, billions of dollars of toxic pesticides are utilized each year and the agents used may pollute water and terrestrial environments for long periods [5].

The increasing uses of fungicides to control the plant pathogenic fungi lead to adverse impacts on human health and the environment [6] The extensive use of fungicides, which to pose more of carcinogenic risk than other pesticides [7] may give rise to undesirable biological effects on animals and human beings [8]. The increasing use of chemical fungicides caused serious environmental problems and harmful non-target organisms and to overcome them must do research to get new and safer pesticides safer. a natural products produced by plants is source of new pesticides.

Any chemical with fungicidal properties could be potentially useful to inhibit fungal growth or speculation. Antifungal compound come from volatile oil producers such tea tree, Eucalyptus, cinnamon, and other [9-11]. Plant oils also have great potential for controlling several fungal pathogens such as *Colletotrichum musae* (Berk &M.A. Curtis) *Arx Lasiodiplodia theobromae* (Pat) Griffon& Maubl. and *Fusarium proliferatum* (Matsushima) Nirenberg [12, 13], and also for controlling many bacterial pathogens [14].

Cinnamon is a bushy, evergreen tree with numerous, long, leathery, bright green leaves, small yellow flowers, and ovoid blackish fruits [11]. Plant oils show antifungal activity against a wide range of fungi [15, 16] Therefore, the purpose of the present study was to evaluate the fungitoxic effect of some plant oils on the fungal pathogens of *Cinnamomum zeylanicum* (Cinnamon)

2. MATERIAL AND METHODS

2.1. Preparation of Plant Oil

Eleven plant oils were tested in the present study; they obtained commercially from two suppliers of 100% pure essential oil (Table 1).

2.2. Oil Suspensions

Oil suspensions were prepared by adding commercial oils to sterile distilled water containing 0.01% Tween 80 [17] The emulsifier Tween 80 was added to enhance the solubility of the oils to obtain concentrations of 0. 1, 0. 2, 0.5,1 and 3 per cent [9, 12, 17].

The range of concentrations of the oils used in the present study 0.1-3%) was based on concentration range adopted from a previous study on tea tree (Melaleucaalternifolia Maiden & Benche) oil and at concentrations that are known to be safe for the host plants (0.04-3%; [18]. Hay and Waterman [10] study showed that oil from basil (Ocimumbasilicum L.) could inhibit the growth of a wide range of fungi at a concentration of 0.15% (v/v), and that the inclusion of 1-10µL/mL of marjoram oil in the culture broth reduced the fungal growth by up to 89% compared with an untreated control. Ranasinghe, et al. [13] found that fungistatic and fungicidal effects of

cinnamon and clove oils against *Colletotrichum musae*, *Lasiodiplodia theobromae*, and *Fusarium proliferatum* within a concentration range of 0.03-0.11%(v/v).

2.3. Synthetic Fungicides

Fungicides chosen for the study were Amistar and Dithane M-45, which are used currently on the pepper farm. The former was obtained from the Syngenta Company and the latter from the L&L Pepperfarm

Amistar is a broad spectrum and systemic fungicide produced by Syngenta. It has an active ingredient of 250 g/L azoxystrobin, which possesses a novel biochemical mode of action: it inhibits mitochondrial respiration in fungi [19]. It is active on all four classes of fungi that attack crops. Today, farmers use Amistar for fungal control in cereals such as wheat and barley as well as in vines, fruits, vegetables, bananas, rice, soy beans, turf and omamentals.

Dithane M-45 is a broad spectrum, contact fungicide produced by Dow Agro Science, Inc. Its active ingredient is 800 g/kg Mancozeb that has a multi-site mode of action that affects many enzymes in the fungi [20]. Dithane M-45 is recommended for control of wide range of diseases. Optimal disease control is achieved when the fungicide is applied in a regularly scheduled, preventative a pray program.

Fungicides usually are mixed on the farms and applied at the rate of 175-210 g/ha of Amister (280 g mixed in 900 L water) and 3-4kg/ha of Dithane M-45 (2 kg mixed in 900 L water).

2.4. Chemical Fungicide

Fungicide concentrations used were selected above and below the usual farm dose rates of 0.31g/L of Amister and 2.22gL of Dithane M-45. The current farm use concentrations (12.5%, 25%, 100%, 2 time, and 5 times). The range of concentrations used was based on a preliminary test. Therefore, the concentrations chosen for further tests were DF_{25} and AF_{100} because spores subjected to DF_{125}, AF_{2x} and AF_{5x} concentrations still germinated after 48 hours, and all of the assessments of the present study were made after 24 hours for ease of operations (Table 2).

2.5. Culture Medium

Czapek-Dox Yeast Extract agar (CDYE agar is the culture medium for fungal isolation and The pH of the medium was adjusted with hydrochloric acid (Hcl) to 4.6 to restrict bacterial growth' appropriate growth media are slightly acid for most fungi, in contrast in the requirements for bacteria, which are generally intolerant of acid conditions. And added to medium chloramphenicol (PH 6,5) [21]. Other culture media such as water agar, potato dextrose agar (PD agar), and potato carrot agar (PC agar) [22] were used at various times throughout the present study in order to stimulate fungal sporulation.

2.6. Fungal Pathogens

Pieces of fresh leaves of Cinnamon plant were cut from diseased and healthy parts of the leaves of plant. These leaf pieces or whole Cinnamon were dipped momentarily in 70% ethanol

and then soaked in a solution of 1% sodium hypochlorite for five or ten minutes. Placed in Petri dishes containing CDYE agar, and maintained in an incubator at 28°C.

2.7. Leaf Microflora

Leaflets (i.e pieces of Cinnamon plant leaves up to 10x10 mm in area) were prepared for assessment of their active fungal populations by senial washing with distilled water [23]. Twelve pieces of 100 mm2 leaflets were placed in Universal bottles for each washing schedule, two replications (Washing schedules were 2, 4, 7, and 10 times). washed by shaking for 2 minutes first with 1 change of 10ml sterile durfactant Tween 80 (2ml/ litre water) using a Griffin Flask Shaker and additional changes of sterile distilled water (10ml). Spread plate assessment of fungal fragment and spores recovered followed the method of Black [24] Finally, 4 pieces of washed leaflets from each washing schedule were plated (leaf plate method, Black [24] onto CDYE sgarin three replication. After incubation for 5 days at 28oc, the plates were examined and the species of each fungus present were counted under a bacterial colony counter Black [24] and identified.

2.8. Identification of Fungi

The fungal colonies growing on the culture plates were identified morphologically on the basis of their colour, type of spores, the presence of sporodocia or appresonia, colony texture, and other growth characteristics of the **fungi** [22].

2.9. Spore Harvesting

The fungal pathogens were cultivated in order to produce viable spores in sufficient numbers Spores were obtained by flooding 1-2 week old cultures with 5 ml of sterile distilled water, and the concentrations of spores were diluted to approximately 50,000/ ml with sterile distilled water [22]. pore age was recorded and spore counts conducted using a haemocytometer [25]

2.11. Germination of Spore

For each fungal pathogen, A germination test was conducted(a period of 24 hours) when germination reached a peak The slides were incubated on humid, senile, Petri plates. Every 4 hours, A count of germination was taken, starting at 6 hours after plating and finishing at 24 hours.

2.12. Testing Methods

Agar (1ml) mixed with 1 ml of spore suspension both at double their final concentrations. Then, 1 ml of the agar-spore suspension was spread over a slide to form a layer and 40mL of plant oil treatment solution (the concentration was 40mL treatment solution/mL agar) was placed on to the surface of agar layer. Then, the slide was incubated in a moist Petri dish.

Results of this tests gave 100% spore germination in distilled water and 91.33%, 86.32%, 85.21% germination.

2.13. Data Analysis

Statistical analysis of the data was undertaken using an SPSS version 11.0 software. The effect of serial washing schedules on fungal colonization was analyzed by one-way analysis of variance and the significance of the differences between means were calculated using the Duncan lest at the 5% level [26].

3. RESULT AND DISCUSSION

3.1. Isolated Fungi from Cinnamon

A total of 11 fungal species were isolated from cinnamon plant (Tables 3).They were identified using information from Bailey and Jeger [27] and Kirk, et al. [28] . Those failing to produce identifiable structures were classified as unknowns. A laboratory code number was given to each fungal a pieces with numbers 1 to 11 allocated to fungi isolated from cinnamon.

11 fungal species were isolated from cinnamon plant, and two fungal genera could not be identified because they failed to sponulate in pure cultures. The identified species of fungi belonged to the genera *Curvularia*, *Helminthosporium*, *Pestalotiopsis*, *Aspergillus*, *Rhizopus*, *Cladosporium*, *Nigrospora* and *Trichoderma*. The fungal genera most frequently isolated from the plant sample were *Cladosporium*, *Curvularia*, *Helminthosporium and Trichoderma*. Members of the genera *Pestalotiopsis*, is commonly pathogenic [5, 29]. Besides being reported as a parasite on other fungi [5, 29]. Aziz, et al. [30] found that Aspergillus and Rhizopus are recognized as the most common contaminant fungi of oil plant.The fungi *Curvularia inaequalis Curvularia sp Helminthosporium* sp, *Pestalotiopsis* sp and *Cladosporium* sp are presumed as a major cause of the disease on cinnamon on account of their frequent occurrence and their known behavior on other host plants.

3.2. Colony Counts

A variety of different organisms, especially bacteria and fungi, were isolated from cinnamon leaves. Fokkema and Van Den Heuvel [31] mentioned that large bacterial populationThe mycelia fungal colonies were counted and the results are given in Table 4. There was a strong trend in the number of isolated fungal colonies to increase from lower to higher washing cycles in all of the plant samples (Table 4). In general, the data show that microorganisms can be washed from a leaf surface, but with some apparent difficulty. The number of colony forming units of fungi recovered increased with the number of washes. While washing presumably releases more microflora from the leaf surface, part of this increase may have been due to the fragmentation of leaves and fungal hyphae. Washing 7 and 10 times did not significantly increase the colonies of microflora recovered from cinnamon. All of the colonies recovered after 10 washes were significantly greater ($P<0.05$) than those found after seven. When extended beyond 10 washing cycles, there was no further increase in fungal recovery.

3.3. Germination of Spore

Germination tests were conducted for each fungal species, over a period of 24 hours in order to determine the best time to spot-sample for peak germination. The times taken for each fungal

pathogen to achieve 80%±5 and 95%±5 germination is summarized in Table 5. *Curvularia inaequalis* fungi reached peak germination (80%±5) within the first seven hours and peak germination level (95%±5) at or after more than 12 hours. Previous experience indicates that where the germination percentage is lower than approximately 70%, the spores should not be used in germination inhibition trials [32]. Fungi of *Curvularia*, *Bipolaris*, *Drechslera*, *Exserohilum* (those formerly included under *Helminthosporium* fungi [29, 33] reached peak germination level within the first seven hours. Meanwhile, other groups of fungi that were most likely to be fungal pathogens reached peak germination level at or after more than 12 hours.

3.4. Effect of Oils on Spore Germination of Cladosporium Sp

The impact of all concentrations from 0.1 to 3% of oils on the germination of *Cladosporium* sp spores are shown in Table 6 Germination of *Cladosporium* sp was 100% in distilled water (DW), 0% in Dithane M-45 fungicide (DF), and 30.2% in Amister fungicide (AF) at 24 hours. The efficiencies of some oils from cinnamon, clove turmeric, and black pepper compared to control treatment of distilled water and synthetic fungicides are shown in Table 6 shows the results of spore germination of *Cladosporium* sp in the presence of all of the oils tested. Oils of tea-tree (TO), eucalyptus (EO), ginger (GO), pepper black (PO), turmeric (TmO), and neem (NmO), at 0.1, 0.2, 0.5 and 3% of concentrations, caused small reductions in germination of *Cladosporium* sp. More noticeable reductions were observed through the application of 0.5-1% concentrations of oils of Limon grass (LgO), Lesser gatangal (GiiesO) and cardamom (CmO), Low concentrations of clove bud and all concentrations of the cinnamon bark produced major reductions in fungal spore germination.

3.5. Effect of Oils on Spore Germination of Fungi from Cinnamon

The results shown in Table 7 indicate that tea-tree oil (TO) significantly inhibited germination of all of the fungi isolated from cinnamon at concentrations of 0.1 or 0.2% with the exception of *Curvularia inaequalis* which responded only at the highest concentration (3%).

The results for lemon grass oil (LgO) shown in Table 8 indicate that complete inhibition of Pestalotiopsis (C21) spore germination occurred at all concentrations tested. Meanwhile, complete inhibition of germination on Curvularia sp. (C30) occurred at 1 or 3 concentration. Significant inhibition of spore germination of the other fungi was noted at oil concentrations of 0.1% or 0.2%. Table 9 show complete inhibition of fungal spore germination, with the exception of those of Pestalotiopsis, by cinnamon barks oil and cinnamon required cinnamon bark oil concentrations above substantially reduce its germination potential.

Germination of spore of fungi from cinnamon subjected to Cinnamon bark oil at a range of concentrations of 0.1 to 3% Table 9 show complete inhibition of fungal spore germination, with the exception of those of Pestalotiopsis, by cinnamon bark oil (all concentration), Pestalotiopsis fungus isolated from cinnamon required cinnamon bark oil concentrations above 0.5 to substantially reduce its germination potential.

Several plant oils have shown remarkable biological activity when tested against fungi [5, 34-36]. While eucalyptus oil and lemon grass oil were reported to inhibit mycelia growth of *Didymella bryoniae* [12]

In the present study, it is clear that several plant oils were active against the germination of spores of fungi isolated from cinnamon. The highest inhibition of spore germination was provided by cinnamon and clove oils in a range concentration of 0.1-3%. These results accord with those **on** Ranasinghe, et al. [13] who showed that these two oils were fungicidal against anthracnose and rot pathogens on bananas. However, the concentration of the oils used in the present study was higher. Many researchers have reported cinnamon and clove oils as good sources of antifungal compounds [13].The concentration of oils that produced significant inhibition of spore germination compared to a distilled water treatment was varied across oils and fungal species. However, many of the oils inhibited spore germination at even the lowest concentrations tested (0.1%). Cinnamon and clove oils, along with other oils such as lemon grass oil, cardamom oil, and lemon myrtle oil in certain concentrations, were more efficient than the synthetic fungicide Amistar at inhibiting spore germination.

The following plant oils tested in the present study were shown to contain antifungal compounds and could produce significant inhibition of germination of fungal spores: cinnamon bark oil, clove bud oil, clove leaf oil, Previous research has shown that cinnamon and clove oils protect against post-harvest fungal diseases on bananas [13].

REFERENCES

[1] D. Persley, *Diseases of fruit crops*. Brisbane: Department of Primary Industries, 1993.

[2] W. E. Fry, *Principles of plant disease management*. San Diego,Califomia: Aczdemic Press, 1982.

[3] C. T. Ingold and H. J. Hudson, *The biology of fungi*, 6th ed. London: Chapman & Hall, 1993.

[4] G. N. Agrios, *Losses caused by plant diseases. Plant pathology*. Oxford, UK: Elsevier, 2004.

[5] G. N. Agrios, *Plant pathology*, 4th ed. San Diego, California: Acadmic Press, 1997.

[6] C. A. Harris, M. J. Renfrew, and M. W. Woolridge, "Assessing the risk of pesticide residues to consumers: Recent and future developments," *Food Additives and Contamination*, vol. 18, pp. 1124-1129, 2001.

[7] Anonymous, *Regulating pesticides in food. The delancy paradox*: National Academy Press, 1987.

[8] K. A. Osman and H. T. Abdulrahman, "Risk assessment of pesticide to human and the environment," *Saudi J. Biol. Sci*., vol. 10, pp. 81-106, 2003.

[9] C. F. Carson and T. V. Riley, "Antimicrobial activity of tea tree oil university of Westem Australia-rural industries research and development corporation, UWA24A-98170," 1998.

[10] R. K. M. Hay and P. G. Waterman, *Volatile oil crops their biology biochemistry and production*. Essex: Longman Scientific & Technical, 1993.

[11] E. A. Weiss, *Essential oil crops*. London: CAB International, 1997.

[12] A. C. G. Fiori, K. R. F. Schwan- Estrada, J. R. Stangartin, J. B. Vida, C. A. Scapim, M. E. S. Cruz, and S. F. Pascholati, "Antifungal activity of leaf extracts and essential oils of some medicinal plants against didymella bryoniae," *Journal of Phytopathology*, vol. 148, pp. 483-487, 2000.

[13] L. Ranasinghe, B. Jayawardena, and K. Abeywickrama, "Fungicidal activity of essential oils of cinnamon zeylanicum (L) and Syzygium aromaticum (L) Merr et I.M. Perry against crown rot and anthracnose pathogens isolated from banana," *Letters in Applied Microbiology*, vol. 35, pp. 208-211, 2002.

[14] H. J. D. Doman and S. G. Deans, "Antimicrobial agents from plants: Antibacterial activity of plant volatile oils," *Journal of Applied Microbiology*, vol. 88, pp. 308-316, 2000.

[15] C. L. Wilson, J. M. Solar, A. Ghaouth, and M. E. Wisniewski, "Rapid evalution of plant extracts and essential oils for antifungal activity against Botrytis cinerea," *Plant Dis*, vol. 81, pp. 201-210, 1997.

[16] M. S. Abd-Alla, K. M. Atalla, and M. A. M. El-Sawi, "Effect of some plant waste extracts on growth and aflatoxin production by aspergillus flavus," *Annala Agric. Sci. Ain Shams Univ. Cairo*, vol. 48, pp. 579-592, 2001.

[17] M. P. Letessier, K. P. Svoboda, and D. R. Walters, "Antifungal activity of the essential oil of hyssop hyssopus officinalis," *Journal of Phytopathology*, vol. 149, p. 673, 2001.

[18] S. Clark and D. Gilrein, "Horticultural oils." Available http:www.cce.comell.edu/Suffolk, 1992.

[19] Anonymous, "Products and services-fungicides syngenta, 2003." Available http:www.syngenta.com [Accessed April 8 2003], 2003a.

[20] Anonymous, "Labels and safely data sheets-dithane M45," *Dow Agro Sciences, 2003."* Available http:www.dowagro.com [Accessed April 9 2003], 2003b.

[21] J. I. Pitt and A. D. Hocking, *Significance of fungi in stored products. In fungi and mycotoxins in stored products*. Canberra: Australian Centre for international Agricultural Research, 1991.

[22] O. D. Dhingra and J. B. Sinclair, "Basic plant pathogen- collototrichum gloeosporioides," *Crop Knowledge Master 1993*, pp. 1-21, 1985.

[23] W. A. Shipton, R. L. MCCown, and W. T. Williams, "Influence of weather on mouldiness and the mycoflora of legume pasture during the dry season in tropical Australia," *Australian Journal of Botany*, vol. 29, pp. 59-69, 1981.

[24] J. G. Black, *Microbiology- principles and explorations*, 4th ed. New Jersey, USA: Prentice-Hall, Inc, 1999.

[25] J. P. Mather and P. E. Roberts, *Introduction to cell and tissue culture. Theory and technique*. New York: Plenum Press, 1998.

[26] J. H. Zar, *Bioststistical analysis*, 4th ed. New Jersey: Prentice Hall international. Inc, 1999.

[27] J. A. Bailey and M. J. Jeger, *Colletotrichum-biology pathology and control*. Wallingford, UnitedKigdom: CAB International, 1992.

[28] P. M. Kirk, P. F. Cannon, J. C. David, and J. A. Stalpers, *Ainsworth & Bisby s-dictionary of the fungi*, 9th ed. Wallingford, United Kingdom: CAB International, 2001.

[29] H. L. Barnett and B. B. Hunter, *IIlustrated genera of imperfect fungi*. Minnesota: Burgess Publishing Company, 1972.

[30] N. H. Aziz, Y. A. Youssef, M. Z. El-Fouly, and L. A. Oussa, "Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins," *Botanical Bulletin of Academia Sinica*, vol. 39, pp. 279-285, 1998.

[31] N. J. Fokkema and J. Van Den Heuvel, *Microbiology of the phyllosphere*. London: Cambridge University Press, 1986.

[32] M. Edman, M. Gustafsson, J. Stenlid, and L. Ericson, "Abundance and viability of fungal spores along a forestry gradient- responses to habitat loss and isolation?," *Oikos*, vol. 104, pp. 35-42, 2004.

[33] Anonymous, "Agar technical (Agar N.3), LP 0013 Oxoid, 2004." Available http:www.oxoid.com/uk/index.asp [Accessed 24 May 2004], 2004.

[34] N. Bhatm, "In vitro evaluation of some leaf extracts agains fusarium spp. Causing yellows of ginger in sikkim," *Plant Disease Research 2001.Cited Available from CABI Abstract 20023033539*, vol. 16, pp. 259-262, 2001.

[35] B. Meena and M. Muthusamy, "Fungitoxic properties of plant extracts against sclerotium rolfsii in Jasmine," *Journal of Ornamental Horticulture (New Series) 2002 [cited 2002]*, vol. 5, pp. 82-83, 2002.

[36] Y. Cohen, A. Baider, B. Ben-Daniel, and Y. Ben-Daniel, "Fungicidal preparations from Inula viscose 2003." Available http://www.infodienst-mir.bwi.de/la/vwo/ecofruvit [Accessed September 9 2003], 2003.

Table-1. Plant oils were tested in the present study

Species	Part of plant used in prepaning	Code
Black pepper (*P.nigrun L*)	Dried unripe berries	PO
Cardamon (*E. cardamomumMaton*)	Seeds	CmO
Cinnamon (*C.zeylanicumBlume*)	Barks and leaves	CnBO
Clove (*SyzygiumBlume*)	Buds and leaves	CiBO, CnLO
Eucalyplus (*Eucalyptus fruticeticum*)	Herbs	EO
Ginger (Z. officinaleRosc)	Rhizomes	GrO
Lemon grass (C.schoenanthus)	Leaves	LgO
Lesser galangal (K galangal L)	Rhizomes	GllesO
Neem (Azadirachtaindica L)	Seeds	NmO
Tea-tree (Melaleucaal temifolia)	Leaves and twigs	TO
Tumeric (Curcuma longa L)	Rhizomes	TmO

Table-2. Germination of C.gloeosporioides responses to different periods of time after incubation and specific synthetic fungicide treatmen

Fungicide (concentration)	Assessment period		
	24 hours	48 hours	72 hours
Dithane M-45 $DF_{12.5}$	Spore germinated	Spore germinated (1-5%)	Spore germinated (no change)
Dithane M-45 DF_{25}	Complete inhibition	Complete inhibition	Complete inhibition
Dithane M-45 DF100	Complete inhibition	Complete inhibition	Complete inhibition
Amistar AF100	Spore germinated	Spore germinated	Spore germinated (100%)
AmistarAF2x Recommended rate	Spore germinated	Spore germinated (89-100%)	Spore germinated (up to 95%)
AmistarAF5x Recommended rate	Spore germinated	Spore germinated (0-3%)	Spore germinated (up to 50%)

Recommended application rate= 2.22g/L
Recommended Recommended rate =0.31g/L

Table- 3. The Isolated fungi from Cinnamon plant

Fungi used in the study	Comments	Laboratory
Curvularia inaequalis**	Cause of leaf spot	C1
Curvularia sp**	Cause of leaf spot	C2
Helminthosporium sp**	Cause of leaf spot	C3
Pestalotiopsis sp***	Cause of leaf blight	C4
Cladosporium sp**	Assessed on pepper	C5
Fungi not used in the study		
Aspergillus sp**	Not a leaf pathogen	C5
Rhizopuss sp**	Not a leaf pathogen	C 6
Nigrospora sp**	Failed to germinate	C8
Trichoderm sp**	Not a leaf pathogen	C9
Unknown species A*	Failed to sponulate	C10
Unknown species B(	Failed to sponulate	C10

** Isolated from serial part
*** Isolated from diseased part and Through serial washing

Table-4. Mean colony –forming units of fungi isolated from cinnamon under several washing cycles.

Plant samples	Washing			
	Number of colony forming units per mL			
Cinnamon	2	4	7	10
	2245	4521	5834	6711

Table-5. Fungi spores' germination assessment over a 24 hour period

Pathogen	Time to germination	
	80%+5	95%+5
Curvularia inaequalis	7	12
Curvularia sp	7	12
Helminthosporiumsp	<7	7
Pestalotiopsissp	20	28

Table-6. Effect of oils on spore germination of C. gloeosporioides at a range of concentrations of 0.1 to 33.

	Germ %				**Std Error**					
Distilled water	100%				0					
DithaneM-45Fungicide treatment	0%				0					
Amistar Fungicide	30.2%				5.1					
Oils	Concentration									
	0.10%		0.20%		0.50%		1%		3%	
	Germ %	Std Error	Germ %	Std Error	Germ %	Std Error	Germ %	Std Error	Germ %	Std Error
Clove bud 0.10%	26%	1.2	22	1.1	6.2	1.2	26	1.1	28	1.3
Tumeric rhizome	82%	2.4	20	0.8	60	2.4	70	5.2	64	1.8
Black pepper berries	85%	2.1	82	2.2	60	2.1	66	1.9	64	2.6
Tea-tree To 0.10%	96%	2.6	90	3.1	92	3.2	88	1.4	86	2.8
Eucalyptus EO 0.10%	96%	2.5	95	2.8	95	3.4	84	1.6	90	4.1
Ginger GrO 0.10%	100%	0	100	0	100	0	98	2.4	98	4.2
Lemon grass LgO 0.10%	100	0	74	1.5	44	1.2	42	3.1	40	1.4
Pepper PO o.10%	88%	2.1	92	2.0	94	3.2	92	3.6	90	3.8
Cinnamon bark CnBO 0.10%	0	0	0	0	0	0	0	0	0	0
Tumeric TmO 0.10%	82%	2.2	74	2.4	72	1.5	68	2.5	66	2.5
Lesser galangal GllesO 0.10%	96	3.1	95	4.1	90	3.9	0	0	0	0
Cardamom Cmo 0.10%	98	2.8	88	2.5	48	1.6	0	0	0	0
Neem NmO 0.10%	88	2.5	92	3.2	84	2.8	84	1.8	90	3.4

Table- 7. Germination of spore of fungi from cinnamon subjected to tea tree oil at a range of concentrations of 0.1 to 3%

TO concentration	***Pestalotiopsis*sp**		**Curvularia inaequalis**		***Helminthosporium*sp**		**Curvularia sp**	
	Germ %	Std Error	Germ %	Std Error	Germ %	Std Error	Germ %	Std Error
DW	88.2	2.8	94.2	1.5	82.4	1.4	100	0
DF	0	0	0	0	0	0	0	0
AF	28.3	4.3	5.2	1.4	20.1	1.7	8.5	1.4
0.1	77.3	1.1	92	2.6	80.2	1.5	80.4	2.3
0.2	65.3	3.5	88	2.5	60	1.5	85.2	2.4
0.5	80.2	7.3	92.1	1.5	65.8	2.2	70.1	2.4
1	15.2	3.7	90.5	1.8	79.5	1.8	72.2	2.4
3	0	0	65.4	4.6	68.5	2.1	40.4	4.2

Table-8. Germination of spore of fungi from cinnamon subjected to lemon grass oil at a range of concentrations of 0.1 to 3%

TO concentration	***Pestalotiopsis*sp**		**Curvularia inaequalis**		***Helminthosporium*sp**		**Curvulariasp**	
DW	Germ %	Std Error	Germ %	Std Error	Germ %	Std Error	Germ %	Std Error
DW	92.2	2.5	94.2	2.2	82.1	0.4	100	0
DF	0	0	0	0	0	0	0	0
AF	30.3	2.6	5.4	3.1	12.4	1.1	7.1	1.2
0.1	0	0	76.2	2.1	51.2	1.6	51.2	3.1
0.2	0	0	66.2	8.2	55.2	2.1	22.2	2
0.5	0	0	40.2	13.2	44.8	0.5	4.1	1.2
1	0	0	25.2	11.2	30.1	1.2	0	0
3	0	0	5.2	1.4	15.2	1.1	0	0

Table-9. Germination of spore of fungi from cinnamon subjected to Cinnamon bark oil at a range of concentrations of 0.1 to 3%

TO concentration	***Pestalotiopsis*sp**		**Curvularia inaequalis**		***Helminthosporium*sp**		**Curvulariasp**	
DW	Germ %	Std Error	Germ %	Std Error	Germ %	Std Error	Germ %	Std Error
DW	91.2	2.1	98.2	1.5	82.1	0.8	100	0
DF	0	0	0	0	0	0	0	0
AF	25.2	4.1	4.5	0	12.2	1.1	7.1	1.1
0.1	55	4.3	0	0	0	0	0	0
0.2	48	3.2	0	0	0	0	0	0
0.5	12.1	4.1	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0