

**Full Length Research Paper**

# A Comparative Study on Aqueous Extracts of Some Indigenous Medicinal Plants and Fungicides against Anthracnose (*Colletotrichum spp*) of Chili (*Capsicum spp*)

Serawit Handiso<sup>a\*</sup> and Tesfaye Alemu<sup>b</sup>

<sup>a</sup>Wolaita Sodo University, Department of Plant Sciences, College of Agriculture, Po Box 138, Wolaita Sodo, Ethiopia

<sup>b</sup>Addis Ababa University, Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences, Po Box: 1176, Addis Ababa, Ethiopia.

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**Corresponding Author:**

**Serawit Handiso**

Wolaita Sodo University,  
Department of Plant  
Sciences, College of  
Agriculture, Po Box 138,  
Wolaita Sodo, Ethiopia.

**Abstract**

Chili (*Capsicum spp*), one of the principal vegetable crop, is being affected by several diseases. Among these, anthracnose caused by *Colletotrichum spp* is the most prevalent one. This research was aimed to manage anthracnose by using different botanical and fungicide antagonist. The treatments were laid in CRD and replicated thrice. The results of the study showed that among aqueous extracts of 8 plant species evaluated the best were aqueous matico extracts, which reduced disease severity by 77.4% followed by Garlic (56.4%), Ginger (48.6%), *Phytolacca dodecandra* (27.28%) and *Desmodium dichotomum* (24.2%). Least growth inhibition of *Colletotrichum* isolates was obtained from *Solanum marginatum* (9.14%). Among fungicides copper oxy chloride (systemic) was found to be most effective which inhibited the growth of the fungus at all three concentrations (0.05, 0.1 and 0.15 percent), that is 86, 94.9, and 98.4% respectively. Similarly 36.24, 47.29, 52.66, 53.96, and 62.2 percent inhibition was observed in case of Mancozeb (non-systemic) at 0.05, 0.1, 0.15, 0.2 & 0.3 percent concentration. Therefore, the utilization of botanicals must be encouraged among small scale poor farmers of Ethiopia.

**Key words:** *Capsicum spp*; *Colletotrichum spp*; fungicides; Matico extract

**Introduction**

Chilli (*Capsicum spp*) is infected by different fungal pathogens, among them anthracnose is the most serious fungal disease and is caused by *Colletotrichum gloeosporioides*. (Shivapuri, et al., 1997; Than et al., 2008; Narasimhan and Shivakumar, 2012). *Colletotrichum gloeosporioides* has also been reported to cause anthracnose in many tropical fruit trees such as mango, papaya (Pandey et al., 2011). This disease is very harmful and can cause spoilage and rotting of fruit plants, resulting in low yield and poor quality of the fruits (Peraza-Sanchez et al., 2005).

Among the large number of diseases affecting chilli cultivation, anthracnose disease caused by *Colletotrichum* species, infection has been most detrimental to chilli production (Than et al., 2008). Anthracnose disease has been reported to be a major constraint in chilli production in tropical and subtropical countries causing huge losses. An estimated annual loss of about 29.5%, amounting whopping figure of US\$ 491.67 million has been reported from India alone (Garg et al., 2014). In India, a calculated loss of 10–54% has been reported in yield of the crop due to the anthracnose disease (Lakshmesha et al., 2005; Ramachandran and Rathnamma, 2006). Significant losses have been reported from other parts of the world as well, like a significant amount of 20–80% loss has been accounted from Vietnam (Don et al., 2007) and about 10% from Korea (Byung, 2007). The loss is high owing to the post and pre harvest involvement of the pathogen causing a loss of 10–80% of the marketable yield of chili fruits (Than et al., 2008). Management of chili anthracnose has been a burning issue for the agriculturists and the farmers as till date, no effective control measures has been proposed. The fall in the chili production and the drop in fruit quality have further intensified the need for developing a sustainable approach for controlling the spread of the disease. No single management technique has been found to efficiently control the disease. Generally, using a combination of the different strategies like chemical control, biological control, physical control and intrinsic resistance has been recommended for managing the disease (Agris, 2005). Numerous reports on the destructive effects of the use of fungicides on farmers' health, economic status, and toxic contamination of the environment, particularly in developing countries cannot be ignored (Voorrips et al., 2004; Garget et al., 2014). Different classes of fungicides have specific mode of action along with their

duration of effect on disease control. So, wise choice of fungicides by the farmers in a particular area, according to prevailing environmental conditions, should be taken into consideration. Rotation of two or more different classes of fungicides is highly recommended for increasing the chance of better protection against the disease in the fields (Förster *et al.*, 2007). Disease control through the use of botanicals extracts of medicinal plants have been explored in recent years for their effective antifungal and antimicrobial properties. Their easy decomposition, non-residual activity and non-phytotoxic properties further popularize their use for controlling phytopathogens. Several studies using crude plant extracts have also been conducted to access the control of *Colletotrichum* spp. on chili (Ngullie *et al.*, 2010; Johnny *et al.*, 2011).

The objective of this research work is to study the effect of some plant extracts and fungicides on management of anthracnose disease of chili caused by *Colletotrichum* spp under in vitro condition with an aim to develop a safe and effective product against chili anthracnose.

## Material and methods

### Pathogen Culture

*Colletotrichum* spp was cultured for 1 week on Potato Dextrose Agar (PDA) at 25 °C. The isolate was obtained from infected chili fruit, leave and stem. Spores were harvested by adding 3-4 ml of sterile distilled water to the petri dish. The spores were then rubbed with a sterile glass rod to free them from the PDA medium and the suspension was passed through two layers of cloth. The suspension was diluted with water to obtain spore concentration of 10<sup>6</sup> spores/ml was determined with Haemocytometer.

### Management of *Colletotrichum* spp isolates

#### In Vitro Evaluation of Botanical Plants against Anthracnose of chili

Plant based bio-pesticides which were relatively economical, safe and nonhazardous can be used successfully against the plant pathogenic fungi. The present investigation was aimed to study the antifungal activity of some plant extracts were evaluated against *Colletotrichum* spp of chili. These botanical plants were selected, that are used for traditional medicine for different as antibiotic in Ethiopia.

**Table 1:** Plant extracts used for the control of plant pathogenic fungi

Sr. No.	Scientific name	Plant part Used	Family	Amharic (Local name)
1	<i>Allium sativum</i>	Bulb	<i>Liliaceae</i>	Nech-shenekurt
2	<i>Zingiber officinale</i>	Rhizome	<i>Zingiberaceae</i>	Zenegeble
3	<i>Phytolacca dodecandra</i>	Leaf	<i>phytolaccaceae</i>	Endod
4	<i>Veronia amygdalina</i>	Leaf	<i>Asteraceae</i>	Grawa
5	<i>Solanum marginatum</i>	Fruit	<i>Solanaceae</i>	Embuay
6	<i>Desmodium dichotomum</i>	Leaf	<i>Fabaceae</i>	Etsfaris
7	<i>Crepis rueppellii</i>	Leaf	<i>Asteraceae</i>	Mucha
8	<i>Piper augustifolium</i>	powder	<i>Piperaceae</i>	-

### Preparation of Cold Aqueous Extract

Fresh plant material were collected and washed first in tap water and then in distilled water. Hundred grams of fresh sample was chopped and then crushed in a surface sterilized pestle and mortar by adding 100 ml sterile water. The extract was filtered through two layers of muslin cloth. Finally the filtrate that was obtained during the extraction used as stock solution (Begum and Bhuiyan, 2006).

### Effect of Botanical Plant Extracts on Mycelial Growth of *Colletotrichum* Isolates

To study the antifungal mechanism of plant extract, the poisoned food technique was used (Begum and Bhuiyan, 2006). ten ml of stock solution was mixed with 90 ml of sterilized molten PDA media, so as to get 10 percent concentration. The medium was thoroughly shaken for uniform mixing of extract.

Twenty ml of poisoned medium was poured into each of the 90 mm sterile Petri plates. Each plate was seeded with mycelium of five mm size discs from periphery of actively growing culture were cut out by cork borer and one such disc was placed at the centre of each agar plate. Controls were also maintained by growing the pathogen on PDA plates. Then such plates were incubated at 25°C temperature for four days and radial growth was taken when maximum growth was occurred in the control plates. Matico that has been taken from Bako agricultural research center used currently as antifungal activity at the concentration of 0.25 % (2500 ppm). 2.5 gm of matico powder diluted with 1 gm of sterilized distilled water and mixed with required amount of PDA. After four days of incubation radial growth was taken when maximum growth was occurred in the control plates. The efficacy of plant extract products was expressed as percent of radial growth over the control which was calculated by using the formula as stated by Sundar *et al.* (1995). Calculating Percent inhibition of mycelial growth over control:

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

Where,

X= Growth of control plate

Y= Growth of fungicide treated plate

### In vitro Evaluation of Matico Powder

Matico was taken from Bako agricultural research center which is currently used. The present investigation was aimed to study the antifungal activity of matico at concentration of 0.25%.

### In vitro evaluation of fungicides

The efficacy of one systemic fungicide and one non-systemic fungicide were assayed at the concentration of 0.05, 0.1, 0.15, 0.2 and 0.3 percent. The fungicides used are given below in table 2.

**Table 2:** Evaluation and testing of systemic and non-systemic fungicide against *Colletotrichum* spp

Sl. No.	Common Name	Chemical Name	Trade Name
1	Mancozeb (non-systemic)	Manganese ethylene bis-dithiocarbonate plus zinc	Indofil M-45 75% WP
2	Copper oxychloride (systemic)	Copper oxychloride	Fytolon 50% WP

Required quantity of individual fungicide was added separately into molten and cool potato dextrose agar in order to get the desired concentration of fungicide. Later 20 ml of the poisoned medium was poured into sterile Petri plates. Mycelial discs of 5 mm size from actively growing culture of the fungus were cut by sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicide to the medium. Each treatment was replicated thrice. Then such plates were incubated at 25°C for four days and radial colony growth was measured. The efficacy of a fungicide was expressed as percent inhibition of mycelial growth over control that was calculated by using the formula suggested by Sundaret al. (1995).

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

Where,

X= Growth of control plate

Y= Growth of fungicide treated plate

## Results

### Management Studies

#### In Vitro evaluation of botanical plants against *Colletotrichum* isolates

The antifungal activity of eight plant extracts, such as, Matico- powder, *Phytolacca dodecandra* fruit, *Desmodium dichotomum* leaf, *Crepis rueppellii* leaf, *Veronia amygdalina* leaf, garlic bulbs, Ginger Rhizome and *Solanum marginatum* fruits were tested against *Colletotrichum* isolates have observed the present inhibition of mycelial growth of the fungal isolates in table 2 and 3. The results in table 3 indicated that considerable inhibition of plant extracts on the mycelial growth of the test fungus was significant. Matico (77.4%) was found effective on inhibiting mycelial growth inhibition which was significantly superior over all other plant extracts evaluated and tested. Garlic (56.4%), Ginger (48.6%), *Phytolacca dodecandra* (27.28%) and *Desmodium dichotomum* (24.2%) were the next best followed by *Crepis rueppellii* (20.01%) and *Veronia amygdalina* (17.2%). Least growth inhibition of *Colletotrichum* isolates was obtained from *Solanum marginatum* (9.14%) (Table 3).

Besides, from the extracts of these seven different botanicals, two plants were thoroughly evaluated invitro against mycelial growth of *Colletotrichum* isolates at 10% of concentration. The results in table 3 indicated that, considerable inhibition of plant extracts on the mycelial growth of the test fungus was significant for garlic and ginger. Both Garlic (*Allium sativum*) and Ginger (*Zingiber officinale*) were found not only highest in inhibiting mycelial growth but also showed extreme difference from other botanicals in percent mycelial growth of *Colletotrichum* isolates inhibition. *Phytolacca dodecandra* (27.28%) and *Desmodium dichotomum* (24.2%) were the next highest mycelial growth inhibition of *Colletotrichum* isolates at 10% of concentration. The second lowest growth inhibition of *Colletotrichum* isolates was obtained from *Phytolaccadodecandra* (17.9%) which is relatively similar to the mycelia growth of *Colletotrichum* isolates treated with *Crepisrueppellii* (20.01%) and *Veronia amygdalina* (17.2%) Matico powder had the peak percent inhibition of mycelial growth of *Colletotrichum* isolates (77.4%) (Table 4).

**Table 3:** In Vitro evaluation of different plant extracts against mycelial growth of *Colletotrichum* isolates at 10% of concentration

No	Plant extracts at Concentration of 10%				Percent inhibition of mycelial growth
	Amharic name	part used	Scientific name	Family name	
1	Endode	Leave	<i>Phytolacca dodecandra</i>	<i>Phytolaccaceae</i>	17.9
	Endode	Fruit	<i>Phytolacca dodecandra</i>	<i>Phytolaccaceae</i>	27.28
2	Mucha	Leave	<i>Crepis rueppellii</i>	<i>Astraceae</i>	20.01
3	Grawa	Leave	<i>Veronia amygdalina</i>	<i>Astraceae</i>	17.2
4	Embuoy	Fruit	<i>Solanum marginatum</i>	<i>Solanaceae</i>	9.14
5	Zenegeble	Rhizome	<i>Zingiber officinale</i>	<i>Zingiberaceae</i>	48.6
6	Nech-shenekurt	Bulb	<i>Allinum satilum</i>	<i>Liliaceae</i>	56.4
7	Etsefaris	Leave	<i>Desmodium dichotomum</i>	<i>Fabaceae</i>	24.2
8	Matico		<i>Piper augustifolium</i>	<i>Piperaceae</i>	77.4

**Table 4:** In vitro evaluation of Matico (*Piper augustifolium*) (Family: *Piperaceae*) leaves powder (0.25%) powder against mycelial growth of *Colletotrichum* isolates

Colletotrichum isolates	Control	Dual culture	Percent inhibition of Mycelia Growth
AU -PEP -1	45	10	77.8
AU PEP -2	75	15	80
AU PEP -6	90	28	68.9
AU PEP -7	85	25	70.6
AU PEP -8	60	12	80
AU PEP -9	40	3	92.5
AU PEP -10	90	25	72.2
Mean	69.3	16.9	77.4

### In Vitro Evaluation of Fungicides

Comparative evaluation of fungicides, namely mancozeb at different concentrations was carried out (Table 5). It was revealed from the finding that the effect of different fungicides on mycelial growth of *Colletotrichum* isolates was significant. Among fungicides copper oxy chloride (systemic) was found to be most effective which inhibited the growth of the fungus at all three concentrations (0.05, 0.1 and 0.15 percent), that is 86, 94.9, and 98.4% respectively. Similarly 36.24, 47.29, 52.66, 53.96, and 62.2 percent inhibition was observed in case of Mancozeb (non-systemic) at 0.05, 0.1, 0.15, 0.2 & 0.3 percent concentration.

**Table-5:** In Vitro Evaluation of Mancozeb Fungicide against Mycelial Growth of *Colletotrichum* Isolates.

Colletotrichum isolates	Fungicides	Mycelial growth Inhibition at different Concentrations (% in ppm)					
		Control	500ppm	1000ppm	1500ppm	2000ppm	3000ppm
AUPEP -1	Mancozeb	45	55.5	60	62.22	64.44	66.67
AUPEP -2	Mancozeb	75	50	50	51.25	52.5	62.5
AUPEP -6	Mancozeb	90	20	33	33.33	33.33	40
AUPEP -7	Mancozeb	85	50	52.5	55	56.25	56.25
AUPEP -8	Mancozeb	60	60	60	60	60	60
AUPEP -9	Mancozeb	40	16.67	73.33	100	100	100
AUPEP -10	Mancozeb	90	0	2.22	5.55	11.11	50
Mean	Mancozeb	62.28	36.02	47.29	52.48	53.95	62.2

In depth analyses on the performance of the *Colletotrichum* isolates at different concentrations of mancozeb gave a different but substantial insight from the management perspective. As indicated in Table 5, it was shown from the finding that the effect of different fungicides on mycelial growth of *Colletotrichum* isolates was significant. Among the different concentrations of mancozeb, the control is found to be inferior in terms of inhibition than the rest of the treatments applied. The most resistant isolates against mancozeb were AAUEP-10, and AAUEP-6 followed by AAUEP-2. However, in isolates AAUEP-10, the trend seemed to be capricious and anomalous. Thus, the trend could not be predicted on the basis of concentration. The overall observation has implied that mycelial growth inhibition capacity increases with increase in mancozeb concentrations. On another experiment that was undertaken to evaluate the mycelial inhibition potential of Copper oxychloride at different concentrations also showed that inhibition efficacy increased with concentration. As exposed in Table 6, it was indicated from the finding that the effect of different concentration of Copper oxychloride fungicides on mycelial growth of *Colletotrichum* isolates was significant. In all three concentration regimes (0.05,

0.1 and 0.15 percent), the magnitudes of mean mycelial growth inhibitions were found to be 86, 94.9, and 98.4% respectively. Similarly 36.24, 47.29, 52.66, 53.96, and 62.2 percent inhibition was observed in case of Copper oxychloride at 0.05, 0.1, 0.15, 0.2 and 0.3 percent concentration. Comparing the efficacy of the two fungicides, copper oxychloride was found to be most effective which inhibited the growth of the fungus at all three concentrations (0.05, 0.1 and 0.15 percent).

**Table 6:** *In Vitro* Evaluation of Copper Oxychloride Fungicide against Mycelial Growth of *Colletotrichum* isolates

<i>Colletotrichum</i> isolates	Concentrations of Copper oxy chloride (in ppm)		
	500ppm	100ppm	1500ppm
AUPEP-1	72.22	95.55	97.78
AUPEP-2	84.37	90.06	96.87
AUPEP-6	93.33	98.67	100
AUPEP-7	93.75	96.25	99.4
AUPEP-8	100	100	100
AUPEP-9	75	95	95.8
AUPEP-10	83.33	88.9	98.89
Mean	86	94.9	98.4

## Discussion

The results on antifungal effect of the aqueous and dry (powder) extracts of seven botanical plants were evaluated against *Colletotrichum* spp under *in vitro* conditions. The botanicals were *Allium sativum*, *Zingiber officinale*, *Phytolacca dodecandra*, *Veronia amygdalina*, *Solanum marginatum*, *Desmodium dichotomum*, *Crepis rupepellii*, and *Piper augustifolium*. Besides, the efficacy of different concentration of Mancozeb (non-systemic) (Manganese ethylene bis- dithiocarbonate plus zinc and Copper oxychloride (systemic) (Fytolon 50% WP) were evaluated. The findings showed that the effect of plant extracts on *Colletotrichum* spp radial growth and disease development vary depending on the type of plant species used, method of extraction, time of application, the type of formulation and part of the plant extracts applied. *Garlic* and *C. procera* were found to be the most effective plant extracts in inhibiting the radial growth of the pathogen *in vitro* condition. However, *Garlic* is the most effective plant extract in reducing the radial growth of *Colletotrichum* spp *in vitro* in both aqueous and dry extracts. The result of this study corresponds with the report by Serawit and Tesfaye, (2014); and Deepka and Padma, (2011) that sprays made from aqueous *Cassia* spp extracts have an antibiotic and antifungal activities/properties. During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics (Zampini, *et al.*, 2009) has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages (Kemo, *et al.*, 2003). Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses (Roopashre, *et al.*, 2008). Arora and Kaur (2007) have studied *Garlic*, exhaustively. However, the use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientists worldwide (Roopashre, *et al.*, 2008; Kunle and Egharevba, 2009; Ettu, *et al.*, 2012). It is an ayurvedic plant with huge medicinal importance. *Garlic* leaf extracts demonstrated antimicrobial activity against both the test bacteria and fungi with water extract (Jaya, 2008).

Prior investigations showed that *Garlic* leaf extracts have antibacterial (Khan and Aslam, 2008). However, not many reports are available on the exploitation of antifungal of antibacterial property of plant products for developing commercial formulations for applications in crop protection. Similar reports made by Tapwal, *et al.*, (2011) on the antifungal properties of some medicinal plants indicating that plant extracts are promisingly vital for the management of *Colletotrichum* spp. In general a voluminous number of literatures reported the effectiveness of *Garlic* against most pathogenic microorganisms. A crude extracts of *Garlic*, however showed the highest inhibitory effect (<10%) at 10% concentrations. The effect of the antifungal compounds may be on spore germination leading to its inhibition of may be due to the effect of these compounds on the cell wall altering the permeability of (Jaya, 2008). The antifungal compounds may also suppress the early stages of mycelia growth leading to inhibition of the fungus. Next to *garlic* extract of ginger had shown 48.00% growth inhibition against *Colletotrichum* spp, followed by *Desmodium dichotomum*. Similar findings on antifungal nature of ginger were documented by Serawit and Tesfaye (2017); Bajwa, *et al.*, (2004); and Sharma and Trivedi (2002). Hence, by utilizing this weed in the plant disease management, one could protect the land from its invasion as well as get some economic gains by disease control. A number of antifungal compounds of diverse skeletal patterns have been found in the plants. These compounds belong mainly to six broad chemical groups, such as phenolics and phenolic acids, coumarins and pyrones, flavonoids, isoflavonoids, steroids and steroidal alkaloids, and other miscellaneous compounds (Mitra, *et al.*, 1984). However, only a few commercial products from the plant are being used in practical plant protection. The fungitoxic effects of the phyto-extracts indicate the potential of selected plant species as a source of natural fungicidal material. Antifungal activity was confirmed by all of the selected plant species and the results revealed that different plant extracts varied in their efficacy for

inhibiting the mycelial growth of tested pathogens. Although the selected concentration of tested plant species was unable to completely inhibit the pathogens but they could be used in combination with the fungicides as IPM strategy to minimize the use of fungicides. The finding of the present investigation could be an important step towards the possibilities of using botanical plant products as pesticides in the plant disease control.

### Conclusion

The findings of this study substantiated that aqueous and dry extracts of *Garlic* have potential to be applied as a control measure against coffee berry disease (*Colletotrichum spp*). The application of aqueous extract of *Garlic* seems promising as it is simple, efficient and inexpensive alternative means of *Colletotrichum spp* management for the farmers, which may replace the synthetic fungicides, particularly to those who cannot afford synthetic chemicals. Likewise, the risk associated with synthetic chemicals as well as consumers' resistance, towards its application in agriculture make the product more attractive natural product for organic agriculture. The findings also provide new scientific information on antifungal activity of *Garlic* against *Colletotrichum spp*.

### Recommendations

Based on the finding of this research, the regional bureau of agriculture is recommended to encourage paper producing application of dry and aqueous extract of *Garlic against chili anthracnose*. For large scale, efficient and cheaper alternative means of *Colletotrichum spp* management for the small scale poor farmers. Government should devise mechanisms to replace the synthetic fungicides for the benefit of those who cannot afford synthetic chemicals. Moreover, awareness must be raised towards the risk associated with synthetic chemicals as well as consumers' toxicity at different levels of food chain. Training must be provided for the farmers regarding pesticide residue, botanical preparation and application against anthracnose. Further, phyto-chemical and antifungal characteristics of botanicals, such as Garlic, Ginger and Matico, against *Colletotrichum spp*. must be strengthened.

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