



EFFICACY OF *Ageratum* LEAF EXTRACT ON POSTHARVEST ROT CAUSED BY *Aspergillus niger* AND *Colletotrichum* sp. ON CHILLI FRUITS

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Abstract: The damage of chilli, an important spice fruit, comes from fungal diseases caused mainly by *Aspergillus niger* and *Colletotrichum* sp. The fungi on chilli fruits would directly harm consumers' health. Plant extracts containing bio-active compounds with antimicrobial properties could be a good possible solution to deal with the fungi. This study aims to evaluate the *in vitro* and *in vivo* efficacy of aqueous extracts from the leaves of *Ageratum* plants against *A. niger* and *Colletotrichum* sp. The results show that the optimal efficacy of the treatment of *Ageratum* leaf extract is at a concentration of 6%, with the efficacy of *Ageratum* leaf extract on colonial diameter at approximately 43-44% for the two fungi at 96 hours after inoculation. The 6% *Ageratum* leaf extract has a high efficacy (~54.23%) on limiting the development of *Aspergillus* rot lesions on chilli fruits 4 days after inoculation. Meanwhile, the efficacy of the extract on *Colletotrichum* lesions is 11.34%, lower than that of *Aspergillus* rot.

Keywords: *Ageratum* leaf extract, chilli fruit, rot lesion

1 Introduction

Chilli is a commonly cultivated spice worldwide with top production countries such as China, Mexico, Thailand, and Turkey [19]. In Vietnam, chilli plants play an important role in the national programme of agricultural transform and rotation. However, losses of chilli production due to diseases and insects are severe from field production to the post-harvest period. Among disease pathogens, *Colletotrichum* sp. and *Aspergillus niger* Tiegh. are important fungal species [7, 15]. The symptoms of infection by *Colletotrichum* sp. on chilli leaves and fruits are characterized by the appearance of yellow to black anthracnose lesions with abundant orange to salmon pink masses of conidia. In the process of infection and invasion, *Colletotrichum* sp. produces various kinds of compounds during germination of spores, development of appressorium, and colonization of chilli tissues, resulting in the breach of host barriers and structural integrity. *A. niger* causes black rot lesions, usually on chilli fruits. Degradation enzymes produced by *A. niger* induce the softening of the flesh tissues of the infected fruits. More seriously, some species of *Aspergillus* could produce mycotoxins, leading to mycotoxicose in humans. Hence, the control of *Colletotrichum* and *Aspergillus* rot is necessary for the chilli

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production in Vietnam. Conventional washing and sanitizing methods even with some of the newer sanitizing agents such as chlorine dioxide and ozone are incapable of reducing fungal populations or preventing disease losses effectively.

There are several traditional strategies developed to manage *Colletotrichum* and *Aspergillus* rot in chilli fruits. First, cold storage or controlled atmosphere storage has been used to delay the ripening process of chilli fruits and thus the decay by anthracnose and *Aspergillus*. The temperature and time depend on the chilli cultivars. However, the disease normally develops when the cold-stored fruits are returned to ambient conditions [1]. Second, the hot water dips to inhibit the mycelial growth and conidial germination of *Colletotrichum* and *Aspergillus* are applied. The high temperature could control the pathogen growth effectively but induces peel darkening on chilli fruits. Third, chemicals such as captan, chitosan, ziram, chlorothalonil, prochloraz, and benomyl for the control of anthracnose and *Aspergillus* rot are exerted [10, 12, 17]. The cost and residue of the chemicals and the ways of application are a major obstacle for disease management on chilli.

In Vietnam, chemical control is currently a common method for chilli fruits, which leads to environmental pollution, toxic remains on fruits, and bad effects on human health. Therefore, the search for plant bio-compounds for managing chilli fruit diseases is underway. A new approach using plant extracts could be a better choice than chemical and physical methods. Pure garlic clove extract completely suppresses the growth of *Aspergillus candidus* and *Aspergillus vesicolor* associated with wheat grains [14], *Aspergillus flavus* on corn [9]. Aqueous extracts from neem seeds, moringa seeds, and garlic bulb at five concentrations of 10, 20, 30, 40 and 50 g/0.25 L show significant differences on radial mycelial growth and weight loss of tomato fruits due to *Aspergillus flavus* [4]. The *Ageratum* leaf extract at different concentrations of 5, 10, 15 and 20% inhibits the *in vitro* growth of *A. niger* on apples. The radial growth of these treatments varies from 80.00% to 91.63%, compared with 93.34% of the control [7]. Extracts of *Magnolia liliflora* Desr. inhibit the radial growth rate of *Colletotrichum capsici* at approximately 61.3% on anthracnose [3]. Besides, plant extracts of *Trida procumbens*, *Jatropha gossypifolia*, *Sida acuta*, *Blighia sapida*, *Ricinus communis*, and *Datura stramonium* have no significant inhibitory effect on conidial germination and sporulation, but the growth rates decrease significantly compared with the control. The Sodom apple fruit extract is effective against root-knot nematodes of chilli plants under glasshouse and field conditions [20]. Meanwhile, the *Ageratum* leaf extract has not yet been applied to disease control on fruits. The objective of this research is to evaluate the efficacy of the *Ageratum* leaf extract on postharvest diseases caused by *A. niger* and *Colletotrichum* sp. on chilli fruits.

2 Materials and methods

2.1 Materials

Fungal isolates of *Colletotrichum* sp. and *A. niger* from chilli fruits were obtained from Department of Plant Protection, College of Agriculture, Can Tho University. Fungi were cultured in Petri dishes containing Potato Dextrose Agar (PDA) for 12 days before performing experiments. The PDA medium was prepared according to Atlas [2].

Fresh mature leaves of *Ageratum conyzoides* L. were collected at a garden of Department of Plant Protection, Can Tho University. The leaves were washed thoroughly twice with tap water and then twice with sterile distilled water, and air-dried at room temperature. A weight of leaves at 2, 4 or 6 g was ground with 100 mL sterile distilled water in a set of sterile mortar and pestle. The macerate was filtered with two layers of Whatman papers.

2.2 Assessment of *Ageratum* efficacy on hyphal development of fungi *in vitro* conditions

The experiments were carried out in a completely randomized design with four treatments including the *Ageratum* leaf extract at three concentrations of 2, 4, and 6% (w/v), and a water control treatment, with six replicates. Separated experiments were performed with *Colletotrichum* sp. and *A. niger*. Experimental steps were conducted according to Falade [5].

A series of *Ageratum* leaf extract solutions was prepared according to the treatment descriptions. Each extract solution was then poured into the PDA medium approximately at 55–60 °C for 2 min with a gentle shake. Ten millilitres of the mixture was immediately poured into Petri dishes using a Dispenser (Effendorf, Germany). After the medium hardened, a hyphal round slice of fungi was put at the center of each Petri dish [5]. The diameter of the fungal colony was recorded at 48 and 96 hours after putting fungal slices. Besides, the rate of fungal spore germination in each extract solution was assessed.

Each experiment was performed 3 times. On the basis of the results of these experiments, the most effective treatment of *Ageratum* leaf extract was chosen to apply to chilli fruits.

2.3 Effect of *Ageratum* leaf extraction on chilli fruits after inoculation with a pathogen suspension

A leaf extract treatment and the water control with 5 replicates were carried out. Separate experiments were conducted with *Colletotrichum* sp. and *A. niger*.

Matured chilli fruits in the experiment have uniform color with no surface damage. They were then washed with tap water once, cleaned with 70% ethanol (v/v), and air-dried at room temperature. Tiny holes with a depth of 2 mm of chilli epidermis were created with a bunch of

four sterile needles. After that, one mL of the fungal spore suspension at a density of 10^6 spores/mL was dropped on these tiny holes. Inoculated chilli fruits were put into an inoculation chamber at 25 °C with a relative humidity of approximately 98% for 24 h. After inoculation, the inoculated chilli fruits were immersed in a solution of *Ageratum* leaf extract for approximately 1 min. Finally, the inoculated chilli fruits were kept in transparent plastic bags at room temperature with wet cotton inside [6, 18]. The lesion length on chilli fruits was recorded at 2, 3 and 4 days after inoculation (DAI).

The efficacy on the reduction of lesion length was calculated using the following formula:

$$\text{Efficacy of extract} = \frac{\text{Lesion length control} - \text{Lesion length of extract treatment}}{\text{Lesion length control}} \cdot 100\%$$

The experiment of each kind of fungi was repeated twice.

2.4 Data analysis

The data were analyzed using Microsoft Office Excel, 2010. The average mean of treatments was compared using Duncan's multiple range test (DMRT) and *t*-test at $p = 0.05$ on the SPSS 16.0 software (IBM, USA).

3 Results and discussion

3.1 Efficacy of *Ageratum* leaf extract against *A. niger* under *in vitro* and *in vivo* conditions

Efficacy of Ageratum leaf extract against Aspergillus hyphal development in vitro

Each tested concentration of *Ageratum* leaf extracts shows different inhibited efficacy under *in vitro* conditions (Table 1). The individual concentrations vary during two observing time points. The optimal efficacy is found at a concentration of 6%. After 96 hours of fungal administration, the colonial diameter at this concentration is 48.23 mm, significantly lower than that of the control (84.97 mm), and the efficacy is 43.24%. Besides, this treatment inhibits the germination of *Aspergillus* spore at approximately 55.77%, statistically different from that of the control (99.67%).

Table 1. Efficacy of *Ageratum* leaf extract on *Aspergillus niger* *in vitro*

Treatment	Germination rate of spore	Colonial diameter (mm) of <i>Aspergillus</i> after putting fungal slices at hour		Efficacy (%) of <i>Ageratum</i> leaf extract on colonial diameter of <i>Aspergillus</i> after putting fungal slices at hour	
		48 ^{1/}	96 ^{1/}	48 ^{1/}	96 ^{1/}
<i>Ageratum</i> leaf extract 2%	84.53 ± 10.3 ^b	34.13 ± 1.3 ^b	52.23 ± 0.6 ^b	32.19 ± 2.1 ^b	38.53 ± 8.3 ^b
<i>Ageratum</i> leaf extract 4%	75.73 ± 15.3 ^c	32.77 ± 0.3 ^b	51.23 ± 1.6 ^b	34.89 ± 8.6 ^b	39.71 ± 11.6 ^b
<i>Ageratum</i> leaf extract 6%	55.77 ± 11.7 ^d	29.77 ± 0.7 ^c	48.23 ± 1.3 ^c	40.85 ± 4.3 ^a	43.24 ± 5.3 ^a
Water control	99.67 ± 3.3 ^a	50.33 ± 1.7 ^a	84.97 ± 2.3 ^a	0.00 ± 0 ^c	0.00 ± 0 ^c
Significance	*	*	*	*	*
CV (%)	11.13	15.22	11.52	15.297	17.12

^{1/}Mean ± SE (standard error) followed by the same letter does not differ significantly according to DMRT at $p \leq 0.05$; *: significant at $p \leq 0.05$

Efficacy of Ageratum leaf extract 6% on chilli fruits after inoculation with A. niger

The *Ageratum* extract considerably reduces the length of rot lesions on chilli fruits. The disease incidence is 100%. At 2 DAI, the lesion length is approximately 0.81 cm, significantly lower than that of the control (1.03 cm) (Table 2). However, the efficacy of treating the *Ageratum* extract is low at 21.36% (Table 2). At 3 and 4 DAI, the *Ageratum* extract significantly inhibits rot lesions. Chilli rotting is nearly isolated, leading to a lesion length of approximately 0.90 and 0.92 cm at 3 and 4 DAI, respectively. These values are statistically different from those of the control (1.48 and 2.01 cm) (Table 2). Moreover, its efficacy gradually increases from 39.18% at 3 DAI to 54.23% at 4 DAI (Table 2, Figure 1A and 1B).

Table 2. Efficacy of *Ageratum* leaf extract on *Aspergillus* rot lesion (cm) of chilli fruits

Treatment	Disease incidence	Length (cm) of <i>Aspergillus</i> rot lesion after inoculation at day			Efficacy (%) of <i>Ageratum</i> leaf extract on chilli fruits after inoculation at day		
		2 ^{1/}	3 ^{1/}	4 ^{1/}	2 ^{1/}	3 ^{1/}	4 ^{1/}
<i>Ageratum</i> leaf extract 6%	100.00 ± 0	0.81 ± 0.09 ^b	0.90 ± 0.10 ^b	0.92 ± 0.08 ^b	21.36 ± 3.4 ^a	39.18 ± 11.5 ^a	54.23 ± 17.5 ^a
Non-treated control	100.00 ± 0	1.03 ± 0.07 ^a	1.48 ± 0.13 ^a	2.01 ± 0.12 ^a	0.00 ± 0 ^b	0.00 ± 0 ^b	0.00 ± 0 ^b
Significance	ns	*	*	*	*	*	*
CV (%)	9.17	23.34	14.57	14.23	15.27	17.12	14.58

^{1/}Mean ± SE (standard error) followed by the same letter does not differ significantly according to *t*-test at $p \leq 0.05$; ns: non-significant at $p \leq 0.05$; *: significant at $p \leq 0.05$

3.2 Efficacy of *Ageratum* extract against *Colletotrichum* sp. under *in vitro* and *in vivo* conditions

Efficacy of Ageratum extract against Colletotrichum hyphal development in vitro

The *Ageratum* extract at a concentration of 6% considerably inhibits the hyphal development of *Colletotrichum* sp. during the observing period. This treatment has an efficacy of 36.03 and 44.03% at 48 to 96 hours, respectively after the administration of fungi. Moreover, the germination rate of *Colletotrichum* spores of this treatment is 67.77%, statistically lower than that of the control (93.33%) (Table 3). Therefore, this concentration of *Ageratum* extract was chosen for the next experiments.

Table 3. Efficacy of *Ageratum* leaf extract on *Colletotrichum* sp. *in vitro*

Treatment	Germination rate of spore	Colonial diameter (mm) of <i>Colletotrichum</i> after putting fungal slices at hour		Efficacy (%) of <i>Ageratum</i> leaf extract on colonial diameter of <i>Colletotrichum</i> after putting fungal slices at hour	
		48 ^{1/}	96 ^{1/}	48 ^{1/}	96 ^{1/}
<i>Ageratum</i> leaf extract 2%	86.77 ± 18.73 ^b	35.77 ± 1.83 ^b	51.43 ± 1.46 ^b	17.55 ± 7.2 ^c	36.78 ± 7.5 ^b
<i>Ageratum</i> leaf extract 4%	76.63 ± 12.66 ^b	33.13 ± 2.43 ^b	51.13 ± 2.23 ^b	23.55 ± 3.9 ^b	37.15 ± 11.8 ^b
<i>Ageratum</i> leaf extract 6%	67.77 ± 13.63 ^c	27.77 ± 1.73 ^c	45.57 ± 1.66 ^c	36.03 ± 5.7 ^a	44.03 ± 9.2 ^a
Water control	93.33 ± 17.66 ^a	43.33 ± 1.56 ^a	81.33 ± 1.56 ^a	0.00 ± 0 ^d	0.00 ± 0 ^c
Significance	*	*	*	*	*
CV (%)	7.01	21.15	12.24	19.38	12.23

^{1/}Mean ± SE (standard error) followed by the same letter does not differ significantly according to DMRT at $p \leq 0.05$; *: significant at $p \leq 0.05$

3.2 Efficacy of *Ageratum* extract on chilli fruits after inoculation with *Colletotrichum* sp.

The efficacy of the *Ageratum* extract was evaluated *in vivo* on the length of anthracnose lesions and the inhibition rate was calculated. The disease incidence of chilli fruits is 100%. At 2 DAI, the lesion length of *Ageratum* treatment is 1.03 cm, not statistically different from that of the control (1.08 cm), and its efficacy is low at 4.63%. At 3 and 4 DAI, the *Ageratum* extract inhibits the development of anthracnose with a lesion length at approximately 1.40 and 1.72 cm, respectively, significantly lower than those of the control (1.58 and 1.94 cm). However, the efficacy is low at approximately 11% (Table 4, Figure 1C and 1D).

Table 4. Efficacy of *Ageratum* leaf extract on anthracnose lesions of chilli fruits

Treatment	Disease incidence	Length (cm) of anthracnose rot lesion after inoculation at day			Efficacy (%) of <i>Ageratum</i> leaf extract on chilli fruits after inoculation at day		
		2 ^{1/}	3 ^{1/}	4 ^{1/}	2 ^{1/}	3 ^{1/}	4 ^{1/}
<i>Ageratum</i> leaf extract 6%	100.00 ± 0	1.03 ± 0.07	1.40 ± 0.11 ^b	1.72 ± 0.14 ^b	4.63 ± 0.13 ^a	11.39 ± 0.28 ^a	11.34 ± 0.32 ^a
Non-treated control	100.00 ± 0	1.08 ± 0.04	1.58 ± 0.12 ^a	1.94 ± 0.09 ^a	0.00 ± 0 ^b	0.00 ± 0 ^b	0.00 ± 0 ^b
Significance	ns	ns	*	*	*	*	*
CV (%)	7.43	15.36	12.17	17.22	16.33	14.27	15.69

^{1/}Mean ± SE (standard error) followed by the same letter does not differ significantly according to *t*-test at $p \leq 0.05$; ns: non-significant at $p \leq 0.05$; *: significant at $p \leq 0.05$



Figure 1. Efficacy of *Ageratum* extract 6% at 4 DAI. A. Control treatment of *Aspergillus* rot lesion; B. *Ageratum* treatment of *Aspergillus* rot lesion; C. Control treatment of anthracnose rot lesion; D. *Ageratum* treatment of anthracnose rot lesion

The efficacy of the *Ageratum* leaf extract is reported by previous researchers [7, 16]. The inhibition zone observed when the aqueous *Ageratum* extract was administered is 8.33 mm at 48 hours on *Aspergillus* [16]. Hidangmayum and Singh [7] show that the *in vitro* efficacy of the 5% and 20% *Ageratum* extract is 1.83% and 14.29%, respectively. However, the *Ageratum* extract has not been applied to the fruits. Therefore, the findings of the current study are novel. In the past studies, the *Ageratum* leaf extract also highly inhibits the growth of bacterium *Xanthomonas campestris*, *Agrobacterium rhizogenes*, and fungus *Aspergillus fumigatus* [2], *Penicillium italicum* [4], *Fusarium oxysporum* f. sp. *lycopersici* [13]. Ilondu et al. [8] study the biochemical composition of the *Ageratum* plant. They report that the *Ageratum* plants contain 2,4,6-tri-tertbutyl phenol (C₁₈H₃₀O, 12.14%), 7-t-butyl-3,3-dimethyl-1-indanone (C₁₅H₂₀O, 9.88%), 1(2H)-naphthalenone-

(1,1-dimethylethyl)-3,4-dihydro ($C_{14}H_{18}O$, 9.19%) and demethoxyageratochromene ($C_{12}H_{14}O_2$, 8.60%). Moreover, other bio-chemicals are present in a very small amount, such as methyl-2-butenic acid ($C_5H_8O_2$, 4.92%), glycerin ($C_5H_8O_2$, 3.0%), 2-methoxy-4-vinyl phenol ($C_9H_{10}O_2$, 6.63%), caryophyllene ($C_{15}H_{24}$, 4.58%), alpha-muurolene ($C_{15}H_{24}$, 5.16%), beta-sesquiphellandrene ($C_{15}H_{24}$, 3.39%), caryophyllene oxide ($C_{15}H_{24}O$, 3.86%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol ($C_{20}H_{40}O$, 3.34%), palmitic acid ethyl ester ($C_{18}H_{36}O_2$, 4.33%), palmitic acid ($C_{16}H_{32}O_2$, 3.59%), phytol ($C_{20}H_{40}O$, 3.49%), linolenic acid ethyl ester ($C_{20}H_{34}O_2$, 3.89%), 5-benzamido-4-oxo-6-phenylhexanoic acid ($C_{19}H_{19}O_4$, 5.16%), and palmitin-2-mono ($C_{19}H_{38}O_4$, 4.82%). The role of each biochemical ingredient of *Ageratum* plants has not yet been elucidated.

4 Conclusion

The *Ageratum* leaf extract at a concentration of 6% effectively inhibits the rot caused by *A. niger* on chilli fruits. The leaf extract highly inhibits fungal development *in vitro* as well as isolates lesions, therefore the rot on chilli fruits surface develops slowly. In addition, this kind of leaf extract could limit the anthracnose disease caused by *Colletotrichum* sp., but its efficacy is low. This research would be crucial if the effective biochemical composition of the *Ageratum* leaf extract is studied in the future.

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