



## A review of application of natural products as fungicides for chili

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### Abstract

Anthraco disease in chillies is a serious problem for farmers. So far, synthetic fungicides have been used as solution for the treatment of this disease. However, the side effects of synthetic fungicides to public health and environment raised awareness on alternative fungicides derived from natural resources. This paper aims to review plants that are potential as an alternative to fungicides for chili plantation, fabrication of test solutions, *in vitro* and *in vivo* fungicide test. Many plants were investigated as alternatives to plant-based fungicide. The utilization of leaves as samples including rhizomes, roots, tubers, weevils, seeds, fruit, flowers and other parts of the plant. The extract fabrication method used as a fungicide test include: maceration method, gradual fractionation method, and decoction method. The maceration method is the method most widely used to extract fungicidal active compounds from plants. Some studies that carried out *in vitro* tests were unable to compare with synthetic fungicides so it was not possible to determine their effectiveness for plant-based fungicide for chillies when compared to synthetic fungicides. *In vitro* extract of 80% alcohol and 10%/60% n-hexane of pacar cina (*Aglaia odorata* L.) leaves can be compared with the performance of propineb 0.2%. In addition, the 60% and 70% kirinyuh (*Chromolaena odorata* L.) leaf extracts were also able to match Acrobat 0.2% performance *in vitro*. Based on the *in vivo* test, suren (*Toona sureni* Merr) leaf extract and nut bulbs can be used as an alternative to vegetable / natural fungicides to help overcome the problem of anthracnose in chillies.

### Keywords :

Chili anthracnose disease, *in vitro* test, *in vivo* test, natural fungicide

## 1 Introduction

One of the goals of the Sustainable development goals (SDGs) is to achieve food security and declare as sustainable agriculture. Chili is one of the food commodities whose production must be increased in order to realize food security in Indonesia. Every year, there are increased in demand for chillies which is in line with the growth in population and the development of food industry that require the chillies as raw material (Subagyo et al., 2010). In addition, there is always increase in the price of chili in particular month due to low productivity of chili harvest. The decrease in chili productivity can be caused by pests and plant diseases (Warisno Dahana, 2018). The pests attack the plants and causes chillies suffered severe damage and crop failure. The pests that can attack chili plants include: peach aphids, thrips pests, mites, fruit fly pests, and fruit borer pests. On the other hand, chili plant diseases include: anthracnose, phytophthora rot, fusarium wilt, cercospora leaf spot, bacterial wilt, yellow virus, mosaic disease (Piay et al., 2010). Therefore, control of plant pest organisms must be done in order to increase the production of chillies (Badan Pusat Statistik Republik Indonesia, 2019).

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Anthraco disease is a red chili plant disease caused by 2 types of fungi, namely: *Colletotrichum capsici* and *Colletotrichum gloeosporioides*. *Colletotrichum capsici* population is fewer than *Colletotrichum gloeosporioides*. The *Colletotrichum capsici* fungus attacks ripen chillies that are reddish in color, while *Colletotrichum gloeosporioides* which has 2 strains, namely: the R strain which only attacks ripe red chillies and the G strain which can attack all parts of the plant, including mature red chillies and those that are still unripe and green. These two types of pathogens are seed-borne diseases because they are able to survive in the seeds for a long time to form acervulus (Piay et al., 2010).

The use of chemical or synthetic pesticides is the most common control. Some examples of synthetic pesticides included: Pyraclostrobin, Azoxystrobin, Picoxystrobin, Difenoconazole, Thiophanate-methyl, Mancozeb (Gao et al., 2017), Metalaxyl-M (Esyanti et al., 2020), Orion 72 WP, Indofil Z-78 WP, Metarial 72 WP, Proven 250 EC, Folicure 5 EC, Propicon 250 EC, Fuji one 40 EC, Flowin HT, dan Winner 250EC (Naznin et al., 2016). The negative impact of usage chemical / synthetic fungicides continuously includes: 1) polluting / damaging the environment, 2) causing residues on plants thus endanger health, and 3) causing resistance on pathogens (Amelia et al., 2020). Therefore, to overcome the negative impact of usage synthetic fungicides, plant-based / natural fungicides can be used. The advantages of natural fungicides include: 1) relatively more environmentally friendly and safe for humans because they are made from natural materials that are easily biodegradable, and 2) cheaper, easy to obtained and easy to applied.

Some plants that have the potential to be used as natural pesticides include: tembelekan/cherry pie (*Lantana camara*), jarak tintir/coral plant (*Jatropha multifida*), pacar cina/chinese rice (*Aglaiia odorata* L.), mengkudu/noni (*Morinda citrifolia* L.), mimba/neem (*Azadirachta indica* A. Juss.), kenikir/compositae (*Cosmos caudatus* Kunth.), sirih/betel (*Piper batle* L.), awar-awar (*Ficus septica*) and others. Basically, natural pesticides do not only come from plants, but also from bacteria, viruses, and fungi (Novizan, 2002). The purpose of this paper is to review: 1) plants that have the potential as an alternative natural fungicide for chili, 2) fabrication of solution for in vitro and in vivo test, 3) in vitro test as fungicide for chili, and 4) in vivo test as fungicide for chili.

## 2 Potential plants as alternative fungicide for chili

Many plants have been investigated on the potential as an alternatives to plant-based / natural fungicides for chili. Table 1 shows the names and parts of the plant and the method tested for fungicide. The part of plants that is widely used in research on finding alternative natural fungicides is the leaves. Few studies have used parts of rhizomes, roots, tubers, weevils, seeds, fruit, flowers or all parts of a plant (combination of flowers, leaves, stems, roots, and seeds). Betel leaf is a part of the plant that has been investigated

both *in vitro* and *in vivo*. The researchers only used one plant type separately to determine its potential as natural fungicide. Only few researchers have combined 2 plants, for example: mixture of betel and tobacco leaf (Oktarina et al., 2017), (Anjani, 2018), (Nur Rohmah, 2017) and mixture of kenikir/compositae (*Cosmos caudatus* Kunth.) and betel (Maimunah et al., 2019).

In general, fungicide test methods used in many studies are divided into 2 categories, namely: 1) *in vitro* and 2) *in vivo*. There are researchers who only focus on using in vitro test methods or in vivo test methods. In addition, the researchers also used both test methods in combination. In the in vitro test method, many types of fungi that cause Anthracnose disease in chilies are used, for example: *Collectotrichum capsici*, *Colletotrichum gloesporioides* and *Colletotrichum acutatum* mushrooms. Several parameters that can be observed in the *in vitro* test include: percentage of inhibition, diameter of fungal colony growth, zone of inhibition, spore growth, spore germination and percentage of spore density. On the other hand, in the *in vivo* test more parameters can be observed which include: anthracnose disease severity, intensity of fungal attack on chilies, percentage of disease incidence, effectiveness of fungicides, diameter of chili spots, incubation period of fungi in chilies, plant height, number of fruit and the weight of the chilies. In this *in vivo* test, the success of the research is strongly influenced by environmental factors, for example: temperature, humidity and rainfall (Suwastini et al., 2020).

**Table 1** Plants that have the potential as alternative fungicide for chili

Name of Plant	Scientific Name	Part of Plant	Test Method for Fungicide	Reference
Umbi Teki	<i>Cyperus rotundus</i> L.	Leaves	In vivo	(Sihite et al., 2020)
Urang Aring	<i>Eclipta alba</i> (L.) Hassk	-	In vitro	(Andreas et al., 2018)
Ketepeng Cina	<i>Cassia alata</i> Linnaeus	All parts of plant	In vitro	(Arneti & Sulyanti, 2017)
Forest Betel	<i>Piper aduncum</i> L.	Leaves	In vitro and In vivo	(Elfina, 2015)
Fragrant Lemon-grass	<i>Cymbopogon nardus</i> L.	Leaves	In vitro and In vivo	(Elfina et al., 2016)
Tobacco	<i>Nicotiana tabacum</i> L.	Leaves	In vitro	(Isman Duila, 2017)
Mixture of Betel and Tobacco	<i>Piper betle</i> L. dan <i>Nicotina tobacum</i>	Leaves	In vitro and In vivo	(Oktarina et al., 2017)
Kunyit	<i>Curcuma longa sensu</i> Val	Rhizome	In vitro and In vivo	(Sari et al., 2020a)
Temu Putih	<i>Curcuma zedoaria</i> (Berg.) Roscoe			
Temu Hitam	<i>Curcuma aeruginosa</i> Roxb			
Putri Malu	<i>Mimosa pudica</i> L.	Root	In vitro and In vivo	(Septianing Ratri, 2017)
Soursop	-	Leaves	In vivo	(Zulkipli et al., 2018)
Betel	-	Leaves	In vivo	(Zulkipli et al., 2018)
Papaya	-	Leaves	In vivo	(Zulkipli et al., 2018)
Garlic	-	Tubers	In vivo	(Zulkipli et al., 2018)
Jarak Tintir	<i>Jatropha multifida</i>	Leaves	In vivo	(Suwastini et al., 2020)
Tembelekan	<i>Lantana camara</i> H. suaveolens (L.) Poit	Leaves	In vitro	(Chatri & Mansyurdin, 2015)
-				
Karamunting	<i>Melastoma malabathricum</i> L.	Leaves	In vitro and In vivo	(Suyanti et al., 2020)
Purun Tikus	<i>Eleocharis dulcis</i>			
Kirinyuh	<i>Chromolaena odorata</i> L.			
Noni	<i>Morinda citrifolia</i>	Leaves	In vivo	(Marsuni, 2020)
Betel	<i>Piper batle</i> L.	Leaves	In vivo	(Juniar Dwi Cahya, 2019)
Tagetes	<i>Tagetes erecta</i>	Leaves	In vitro	(Satryawibowo et al., 2015)
Suren	<i>Toona sureni</i> Merr.	Leaves	In vivo	(Andriyani et al., 2020)
			In vitro	(Andriyani & Purwantisari, 2019)
Betel	<i>Piper betle</i>	Leaves	In vitro and In vivo	(Trisnawati, D., Nugroho, L. P. E., 2019)

Fragrant Lemon-grass	<i>Cymbopogon nardus</i> L.	Leaves	In vitro and In vivo	(Syabana et al., 2015)
Papaya	<i>Carica papaya</i> Linnaeus	Leaves	In vitro	(Liswarni & Edriwilya, 2020)
Pacar Cina	<i>Aglaia odorata</i> L.	Leaves	In vitro and In vivo	(Efri et al., 2017)
Neem	<i>Azadirachta indica</i> A. Juss	Juss	Leaves In vivo	(Aziziy et al., 2020)
Kepok Banana	-	Hump		
Noni	<i>Morinda citrifolia</i> L.	Leaves	In vitro	(Anggreini et al., 2016)
Neem	<i>Azadirachta indica</i> A. Juss.			
Kenikir	<i>Cosmos caudatus</i> Kunth.	Leaves	In vitro and In vivo	(Amelia et al., 2020)
Babadotan	<i>Ageratum conyzoides</i>	Leaves	In vitro	(Wulandari et al., 2015)
Awar-awar	<i>Ficus septica</i>	Leaves	In vitro and In vivo	(Sudirga, 2018)
Mixture of Betel and Tobacco	<i>Piper betle</i> L. dan <i>Nicotina tobacum</i>	Leaves	In vitro and In vivo	(Anjani, 2018)
Gelinggang	<i>Cassia alata</i> L.	Leaves	In vivo	(Supriati et al., 2016)
Jarak Pagar	<i>Jatropha curcas</i> L.	Seed	In vitro and In vivo	(Lestari et al., 2020)
Mixture of Betel and Tobacco	<i>Piper betle</i> L. dan <i>Nicotiana tabacum</i> L.	Leaves	In vitro and In vivo	(Nur Rohmah, 2017)
Binahong	<i>Anredera cordifolia</i>	Leaves	In vitro and In vivo	(Yulia et al., 2019)
Neem	<i>Azadirachta indica</i> A. Juss.	Leaves	In vitro and In vivo	(Ali et al., 2012)
Noni	<i>Morinda citrifolia</i> L.	Fruits	In vitro and In vivo	(Ali et al., 2013)
Betel	<i>Piper betle</i> L.	Leaves	In vivo	(Damiri, 2011)
Noni	<i>M. citrifolia</i>	Fruits	In vitro	(Septiana et al., 2013)
Betel	<i>Piper betle</i> L.	Leaves	In vitro	(Ningtyas et al., 2013)
Babadotan	<i>Ageratum conyzoides</i>			
Noni	<i>Morinda citrifolia</i>	Leaves, Flowers, and Fruits	In vivo	(Efri, 2010)
Jarak	<i>Jatropha curcas</i> L.	Leaves	In vivo	(Wanda et al., 2014)
Mimba	<i>Azadirachta indica</i>			
Betel	<i>Piper betle</i> L.	Leaves	In vivo	(Wati et al., 2014)
Babadotan	<i>Ageratum conyzoides</i> L.			
Babadotan	<i>A. conyzoides</i>	-	In vivo	(Gusmarini et al., 2014)
Siam	<i>C. odorata</i>	-		
Reed	<i>I. cylindrica</i>	-		
Teki	<i>C. rotundus</i>	-		
Camplong	<i>Callophyllum inophyllum</i>	Fruits	In vitro	(Sholehah, 2012)
Patchouli Oil	-	-	In vitro and In vivo	(Sakerebau & Wahyu, 2013)
Ubi Ungu	<i>Ipomoea batatas</i>	Leaves	In vitro and In vivo	(Saputri & Utami, 2020)
Mixture of Kenikir and Betel	<i>Cosmos caudatus</i> dan <i>Piper betle</i>	Leaves	In vitro	(Maimunah et al., 2019)
Putri Malu	<i>Mimosa pudica</i>	Leaves	In vivo	(Eviyanti, 2020)
Kirinyuh	<i>Eupatorium odoratum</i> L.	Leaves	In vitro and In vivo	(Indrawati, 2021)
Cinnamon	<i>Cinnamomum burmannii</i>	Leaves	In vitro	(Darmadi et al., 2021)
Neem	<i>Azardiachta indica</i>	Leaves	In vitro	(Rahman et al., 2019)
Garlic	<i>Allium sativum</i>	Rhizome	In vitro	(Rahman et al., 2019)
Zinger	<i>Zingiber officinale</i> Rhizome			
Termaric	<i>Curcuma longa</i>	Rhizome		
Tulsi	<i>Oscimum sanctum</i> Linn.	Leaves		
Mahogoni	<i>Swietenia mahogoni</i>	Leaves		
Mehendi	Leaves			

**Table 2** Methods of extract preparation for *in vitro* and *in vivo* test

Plant	Method	Solvent	Sample : Solvent (w/v)	Result	Reference
Binahong Leaf	Maceration for 1 x 24 hours then concentrated using rotary evaporator	90% methanol	1 : 4	Sticky	(Yulia et al., 2019)
Banana Hump and Mimba Leaf	The sample was sieved with size of 25 mesh and macerated separately for 1 x 24 hours then concentrated with rotary evaporator to 250 mL	100% methanol	6 : 10	Condensed extract	(Tobing & Mulyaningsih, 2020)
Jarak Pagar Seed	Maceration for 48 hours then concentrated with rotary evaporator	96% ethanol	1 : 3 and 1 : 2	-	(Lestari et al., 2020)
Mixture of Betel and Tobacco Leaf	The sample was separately dried and then crushed by adding distilled water and then filtered	Distilled water	1 : 1	-	(Oktarina et al., 2017)
Leaf of Pasang Surut Weeds	Leaf powder is macerated for 2 x 24 hours then concentrated with rotary evaporator at a temperature of 40 - 70 °C and followed by evaporation process with water bath at temperature of 50 - 60 °C	96% ethanol	1 : 4	-	(Suyanti et al., 2020)
Awar Awar Leaf	Maceration for 72 hours then evaporated with rotary evaporator	Methanol	1 : 10	-	(Sudirga, 2018)
Babadotan Leaf	Graded fractionation	Water, methanol, ethyl acetate, and n-hexane	1 : 10	-	(Wulandari et al., 2015)
Noni Leaves and Fruit	Maceration for 3 x 24 hours then concentrated with rotary evaporator and waterbath at temperature of 60 °C	Methanol	1 : 3	Condensed extract	(Nurul et al., 2020)
Kenikir Leaf	Maceration for 2 x 24 hours: the first soaking for 6 hours, then stirring it then leaving it for 18 hours then concentrating it with rotary evaporator and then concentrating again with a waterbath at temperature of 40 °C	96% ethanol	1 : 3 and 1 : 2	-	(Amelia et al., 2020)
Noni and neem leaf	Using simple fractionation tool	Water	1 : 5	-	(Anggreini et al., 2016)
Pacar Cina Leaf Graded fractionation	The sample was added with sterile distilled water, blended until smooth and put in sterile erlenmeyer and covered with aluminum foil. extract was heated until boiling and then filtered	Distilled water	1 : 20	-	(Arneti & Sulyanti, 2017)
Distilled water, alcohol, and n-hexane - - (Efri et al., 2017)					
Ketepeng Cina Fragrant Lemongrass Leaf	The sample was heated in water at 90 °C for 30 minutes then concentrated on rotary evaporator	Water	-	Concentrated extract	(Syabana et al., 2015)
Suren Leaf	Maceration for 1x24 hours then concentrated with rotary evaporator	70% ethanol	1 : 3	Pure extract	(Andriyani & Purwantisari, 2019)
Tagetes Leaf	Using simple fractionation tool	Water, methanol, ethyl acetate, and n-hexane	-	-	(Satryawibowo, 2015)
Putri Malu Root	The maceration and then evaporated with rotary evaporator	Ethanol	9 : 10	Condensed extract	(Eviyanti, 2020)
Jarak Tintir and Tembelekan	The sample was separately extracted using simple fractionation tool and then evaporated with rotary evaporator	Water	1 : 5	Dry extract	(Suwastini et al., 2020)
Neem, Betel, and Clove Leaf	Maceration for 3 days and stirring 3 times a day then concentrated with rotary evaporator	96% ethanol	1 : 5	Concentrated extract	(Sitompul, 2017)

Curcuma spp. Rhizome	Maceration	Methanol	-	Condensed extract	(Sari et al., 2020b)
Urang aring	Maceration for 3 x 24 hours then evaporated with rotary evaporator at temperature of 40 °C	Ethanol	1 : 4	Concentrated extract	(Andreas et al., 2018)
Shallot and Garlic	Extraction and then extracted in centrifuge and filtered	Distilled water	1 : 1	Crude extract	(Sittisart et al., 2017)
Carica papaya L. Cv. Leaf and Maradol Seed	Maceration for 24 hours then the extract was filtered and centrifuged then evaporated with rotary evaporator	Ethanol	1 : 8, 1 : 10, 1 : 12	-	(Chávez-Quintal et al., 2011)
Noni Leaf	Using multilevel extraction	Water, alcohol, ethyl acetate	1 : 5	Dry extract	(Putra, 2017)
Galangal Rhizome, Clove Leaf, and Bangun Bangun	Maceration extraction for 24 hours then evaporated by rotary evaporator	95% ethanol	-	Condensed extract	(Harianto, 2018)

### 3 Preparation of extract

The preparation of test solutions for chilies fungicide was summarized in Table 2. In general, the method of extracts preparation used can be classified into 3 types, namely: 1) maceration method, 2) graded fractionation method, and 3) decoction method.

#### 3.1 Maceration method

This method are most widely used to extract active compounds from certain plants for fungicide. Plants were prepared in the powder or flour form are added with a solvent and then are soaked for a designated time. The filtrate is separated from the dregs and the maceration process can be continued with new solvent until color filtrate is clear. The filtrate is concentrated using rotary evaporator with temperature control according to the type of solvent used until concentrated extract is free solvent (K Ngibad, 2019), (Khoirul Ngibad, 2019), (Wibowo et al., 2019). The solvents used in the extract preparation for test fungicide on chilies, including: water solvent (ultrapure water) and organic solvents (90% methanol, methanol, 70% ethanol, 96% ethanol, ethanol, ethyl acetate, and n-hexane). The usage of solvents in the maceration process is expected to be able to extract the large fraction of possible fungicidal active compounds. In addition, there are differences in the ratio of sample weight and volume of solvent used between researchers, ranging from 1: 1 to 1: 10. The greater the ratio of solvent volume and sample weight will maximize the extract or active fungicidal compound produced. However, it is necessary to pay attention to the effectiveness of usage the solvent volume.

#### 3.2 Stratified fractionation method

Practices of the graded fractionation method have been carried out, for example: the fine powder of Chinese henna leaves was fractionated in stages using filter made of various sizes of paralon to form funnel containing activated charcoal as filter and adsorption of nonpolar compounds. The liquid-liquid solvent extraction method used cold distilled water. Then, it was followed by solution of alcohol or n-hexane with concentrations of 10, 20, 30, 40, 50, 60, 70, 80, and 90%, respectively (Efri et al., 2017). Then, babadotan/goatweed (*Ageratum conyzoides*) leaf powder was placed into simple fractionation tool, then the filtered residue was collected and air-dried. The filtrate or crude extract was added with methanol solvent then was collected and air-dried to obtain the methanol fraction of the babadotan leaf extract. In the same way, to get ethyl acetate and n-hexane extract (Wulandari et al., 2015). The water solvent is expected to be able to extract

the active polar fungicide compound which is polar. Decreasing the level of polarity starting from methanol, ethyl acetate, and n-hexane solvents is expected to be able to separate the active fungicide compounds based on their polarity level.

#### 3.3 Decoction method

Decoction method has been used to extract the fungicidal active compounds found in betel leaf. Samples were boiled in water with ratio of 1: 1 for 1 hour. The extract are filtered and sterilized using autoclave at temperature of 121 °C to obtain sterile betel leaf extract (Trisnawati et al., 2019). The boiling process of the *Cassia alata* Linnaeus sample which was blended with water was carried out for 15 minutes (Arneti Sulyanti, 2017). This decoction method is rarely used because it is feared that the active fungicidal compounds present in the sample could be damaged by heat treatment.

### 4 In vitro test as fungicide for chili

The review results of research related to in vitro fungicide test are summarized in Table 3. The concentration of the test solution was carried out in various ways. For example, the concentration of mixture of betel leaf and tobacco extract with concentration of 30% was made by mixing 7 ml of PDA (Potato Dextrosa Agar) and 3 ml of mixture of betel and tobacco extracts (Nur Rohmah, 2017). Another technique was found in preparation of kenikir leaf extract test solution which is done by mixing the extract with Tween 80 as emulsifier with ratio of 1: 1 (w / v) and diluted using sterile distilled water to get concentration of 5%, 10%, 15%, and 20% (Amelia et al., 2020). In other cases, the suren concentrated extract was assumed to be 100% concentration then the concentrated extract was diluted using distilled water into several concentrations (25%, 50%, and 75%) (Andriyani et al., 2020). Besides water, methanol was also used as solvent to make test solution for the *Curcuma sp.* rhizome with concentration of 4-12 ppm (Sari et al., 2020b).

The synthetic fungicide control used by several researchers in in vitro tests included: propineb 70%, 0.2% propineb, azoxistrobin, diphenconazole, benomyl, anthracol, 0.2% acrobat and 0.2% carbendazim. The usage of synthetic fungicide controls is very useful as comparison against the plants being studied. Many studies do not use synthetic fungicide controls so that the potential of these plants is less known when compared to synthetic fungicide controls. On the other hand, the most widely used fungi for in vitro tests are *Colletotrichum capsici* and then *Colletotrichum gloeosporioides*.

**Table 3** *In vitro* fungicide test on chili test

Plant	Concentration Test Solution	Synthetic Fungicide Control	Type of Mushroom	Test Results	Reference
Jarak Pagar Seed	10 - 40%	-	Colletotrichum capsici	The percentage of inhibition of fungal mycelium (%): 18.90 – 31.08	(Lestari et al., 2020)
Betel and Tobacco	30% with concentration ratio (1: 1), (1: 2), (2: 1), (1: 3) (3: 1)	-	Colletotrichum sp	Percentage of Colletotrichum sp colony inhibition (%): 0.56 - 30.44	(Nur Rohmah, 2017)
Betel and Tobacco	30% with ratio of 3: 1	-	Colletotrichum sp	Average of inhibition power (%): 45.08 - 15.68 Average of spore density (106spores / mL): 15.8 - 35.2	(Anjani, 2018)
Babadotan Seed	-	Propineb 70% 1 g/L	Colletotrichum capsici	<b>Colony diameter of C. capsici</b> Water extract: 99.28% Methanol extract: 76.81% Ethyl acetate extract: 137.20% N-hexane extract: 85.27% Propineb 70%: 0% <b>Number of spores</b> Extract water: $5.33 \times 10^6$ Methanol extract: $2.34 \times 10^6$ Ethyl acetate extract: $7.78 \times 10^6$ N-hexane extract: $4.90 \times 10^6$ 70% propineb: $0.00 \times 10^6$	(Wulandari et al., 2015)
Noni Leaves and Fruit	5%	-	Colletotrichum capsici	Percentage of inhibition (%) Leaves: 2.27 Fruits: 2.78 Amount of conidium (conidium/mL) Leaves: 1.44 Fruits: 1.42	(Nurul et al., 2020)
Kenikir Leaf	5 – 20%	-	Colletotrichum sp	Percentage of inhibition (%) : 10.76 – 41.12	(Amelia et al., 2020)
Pacar Cina Leaf	Aquades extract and 10 - 90% alcohol extract Aquades extract and 10 - 90% n-hexane extract	Propineb 0,2%	Colletotrichum capsici	Growth diameter of C. capsici on day 7 (cm): 5.85 - 1.00 Spore density of C. capsici: 15.77 - 0.88	(Efri et al., 2017)
Papaya Leaf	1 – 5 %	-	Colletotrichum gloeosporioides	Growth diameter of C. capsici on day 7 (cm): 2.16 - 0.5 Spore density of C. capsici: 4.8 – 0 Colony area and effectiveness: 41.32 - 20.11 and 6.13% - 64.04% Mushroom wet weight: 4.69 - 45.16% Mushroom dry weight: 8.33 - 54.16% Number of conidia: 27.5 - 82.5%	(Arneti & Sulyanti, 2017)
Suren Leaf	10 – 30%	-	Colletotrichum capsici	Percentage of inhibition of C. capsici colony diameter: 45.49 - 62.74	(Andriyani & Purwantisari, 2019)
Tagetes Leaf	Water, methanol, ethyl acetate, and n – hexane extracts)	Propineb 70%	Colletotrichum capsici	Percentage of colony diameter: 55.18 - 92.47% Percentage of Spore density: 7.20 - 36.88	(Satryawibowo, 2015)
Pasang Surut Weeds	Extract of puruntikus, extract of kirinyuh, and extract of karamunting	Azoxistrobin, diphenconazole, and benomyl	Colletotrichum sp	The percentage of inhibition: 6.99 - 79.54	(Suyanti et al., 2020)
Essential oil (Hyptis suaveolens L.)	Young leaves 0.5 – 2.5% Mature leaves 0.5 - 2.5%)	-	Colletotrichum gloeosporoides	Percentage of inhibition (%): 50 - 65	(Chatri & Mansyurdin, 2015)
Putri Malu	30 – 90%	-	Colletotrichum sp	Percentage of inhibitory power (%): 14.36 - 28.01 The average of spore density (10 <sup>6</sup> spores / ml): 19.11 - 4.44	(Septianing Ratri, 2017)
Curcuma spp	4 – 12 ppm	-	Colletotrichum capsici	The average of colony growth diameter (cm): 1.50 - 0.58	(Sari et al., 2020b)

Betel and Tobacco Leaf	Biorational extract: (1:1), (1:2), (2:1), (1:3), (3:1)	-	Colletotrichum capsici	Percentage of inhibition of fungal colonies (%): 0.56 - 30.44	(Oktarina et al., 2017)
Tobacco	25 – 100%	-	Colletotrichum sp	Average of spore density ( $10^6$ spores/ ml): 31.9 - 7.6 Percentage of inhibition (%): 6.56 - 33.78	(Isman Duila, 2017)
Flour of fragrant lemon-grass	50 – 250 g/l	-	Colletotrichum capsici	Average of spore density ( $10^6$ spores / ml): 19.57 - 100 Percentage of inhibitory power (%): 17.47 - 34.43	(Elfina et al., 2016)
Ketepeng Cina	5%	-	Colletotrichum gloeosporioides	Percentage of inhibitory power (%) Root: 11.96 Seeds: 17.21 Interest: 30.68 Young leaves: 38.40 Stems: 56.37 Old leaves: 64.30	(Arneti & Sulyanti, 2017)
Urang Aring	5 - 25%	Antracol	Colletotrichum sp	Number of mushroom colonies on day 7: 125.75 - 72.75 Diameter of mushroom colonies on day 7: (1.254 - 1.38)	(Andreas et al., 2018)
Banana Hump and Mimba Leaf	15 – 45%	-	Colletotrichum capsici	Percentage of inhibition zone for fungal colonies: 10.76 - 6.58	(Tobing & Mulyaningsih, 2020)
Cinamon leaf Extract	0,5 - 1,50%	-	Colletotrichum capsici	Percentage of inhibition (%): 17 - 100% Percentage of spore density (%): 51 - 100	(Darmadi et al., 2021)
Leaf Extract of Ficus septica	1 – 5%	-	Colletotrichum acutacum	Colony diameter (mm): 29.72 - 81.39 Percentage of spore density ( $10^5$ spora / ml): 63.21 - 99.11 Percentage of spore density ( $10^5$ spora / ml): 63.21 - 99.11	(Sudirga et al., 2014)
Kirinyuh Leaf Extract	10 – 70%	Acrobat 0,2%	Colletotrichum capsici	Average percentage of fungal colony growth (%): 100.00 - 0.00 Fungal inhibition zone on day 10: 1.59 - 1.10	(Indrawati, 2021)
Mansoa alliacea Extract	1 – 5%	-	Colletotrichum acutacum	Colony diameter (mm): 63.25 - 17.00 Spore growth ( $10^5$ spores / mL): 4.19 - 0 Spore germination ( $10^5$ spores / mL): 2.59 - 0	(Sudirga et al., 2019)
Akar Putri Malu	25 - 100%	-	Colletotrichum sp	Percentage of inhibition zone diameter (%): (70 - 10)	(Eviyanti, 2020)
Neem Seed Kernel Extract	Neem oil, garlic bulb extract, combinate application of neem, garlic, ginger, onion plant extract, and neem seed kernel extract (NSKE)	Carbendazim 0,2%	Colletotrichum capsici	Percentage of inhibition: 74.77-68.75	(Musakhan & Zacharia, 2017)
Purple Sweet Potato	5 – 40%	-	Fusarium sp	Average percentage of inhibitory power (%): 56.7 - 76.6	(Saputri & Utami, 2020)

Some of the parameters used in the in vitro test include: colony diameter, percentage of colony inhibition, density / number of spores, and colony area. Colony diameter is measured by making vertical and horizontal lines perpendicular to each other at the bottom of the petri dish as vertical and horizontal diameters.

Then, the colony diameter is calculated using formula (Andreas et al., 2018) :

$$Colonydiameter(cm) = (D1 + D2)/2 \quad (1)$$

With :

D1 = diameter of horizontal colony

D2 = diameter of vertical colony

Observations are made by measuring the diameter of the growth of *C. capsici* colonies. The measurement of inhibition using the formula:

$$DH(\%) = (a - b) / a \times 100\% \quad (2)$$

With :

DH = Inhibition (percent)

a = diameter of *C. capsici* colony (mm) (negative control)

b = diameter of *C. capsici* colony (mm) (treatment)

Spore density was determined by taking 1 ml of spore suspension from isolate propagation treatment. Furthermore, the spore density was calculated using hemocytometer that had been dropped by the suspension under a double lens (binocular), which is one type of lens from a light microscope with a magnification of 400 times. (Herlinda et al., 2006). The spore density was calculated using Gabriel Riyatno formula (1989) (Gabriel Riyanto, 1989):

$$C = \frac{t}{n \times 0,25} \times 10^6 \quad (3)$$

With:

C = spore density per ml of solution

t = total number of spores in the sample box observed

n = number of sample boxes (5 large x 16 small boxes)

0.25 = correction factor for the use of a small-scale sample box on the haemocytometer

Colony area was measured using millimeter plotting paper by depicting the colony area on plastic glass (Liswarni Edriwilya, 2020). The plants studied as an alternative to natural fungicides for chili have the ability to inhibit the growth of anthrax-causing fungi in chilies by in vitro study, which include: *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, and *Colletotrichum acutatum*. However, many in vitro studies do not compare with synthetic fungicides. So, it is not possible to know the effectiveness of the performance of natural fungicides for chilies when compared to synthetic fungicides. Based on Table 4, it can be seen that 80% alcohol extract and 10% n-hexane extract and 60% Chinese henna leaves is similar with the 0.2% propineb performance by in vitro study. In addition, the 60% and 70% kirinyuh leaf extracts were also able to match 0.2% acrobat performance by in vitro study.

**Table 4** Comparison of effectiveness of natural fungicides for chilies with synthetic fungicides

Plants	Synthetic Fungicides	Explanation	Reference
Babadotan Leaf	70% Propineb	The effectiveness of the three extract fractions < propineb 70%	(Wulandari et al., 2015)
Pacar Cina Leaf	0.% Propineb	The effectiveness of 80% alcohol extract and 10% and 60% n-hexane extract is is comparable to 0.2% propineb	(Efri et al., 2017)
Tagetes Leaf	70% Propineb	Cannot match the effect of 70% propineb	(Satryawibowo, 2015)
Urang Aring	Antracol	Effectiveness of urang-aring < Antracol	(Andreas et al., 2018)
Kirinyuh Leaf Extract	0.2% Acrobat	The effectiveness of 60% and 70% kirinyuh leaf extract is is comparable to 0.2% acrobat	(Indrawati, 2021)
Neem Seed Kernel Extract	0.2% Carbendazim	The effectiveness of neem seed kernel extract < 0.2% carbendazim	(Musakhan & Zacharia, 2017)

## 5 In vivo test as fungicide for chili

In effort to find alternatives natural fungicides, the researchers focused not only on in vitro studies but also in vivo studies of var-

ious plants with certain concentrations as shown in **Table 5**. This in vivo test was directly applied to chili plants to be treated with natural fungicides with test conditions appropriate to the actual environment in chili farm.

**Table 5** In vivo fungicide test for chili

Plants	Concentration of Test Solution	Synthetic Fungicide	Test Parameters	Test Results	Reference
Neem Leaf Extract	5 – 20%	-	Incubation period	9 – 12 days	(Sitompul, 2017)
Betel Leaf Extract	Incubation period	12 – 19 days	The severity of anthracnose	8.84 – 8.43%	(Sitompul, 2017)
Clove Leaf Putri Malu Root Extract	Extract 30 – 90%	-	The severity of anthracnose Incubation period Percentage of disease incidence	8.88 – 7.74% 14 – 14.33 days 62.5 – 0%	(Sitompul, 2017) (Eviyanti, 2018)
Suren Leaf Extract	25 – 100%	Mankozeb 1g/L	Spot diameter Incubation period Percentage and fresh weight of healthy cayenne pepper affected by anthracnose Morphometry of chili fruit	0 mm 12 days 91.16 – 99.68% Length: 7.74 – 8.54 cm	(Andriyani et al., 2020)



					Diameter : 1.14 – 1.48 cm	
Extract of Umbi Teki	5 – 25%	Propineb		Chili plant height The severity of anthracnose The severity of anthracnose	122.8 – 131.6 cm 4 – 0% 13.00 – 17.50%	(Sihite et al., 2020)
Extract of Curcuma Longa Sensu	4 – 12 ppm	-		Plant height Number of fruit Fruit weight Symptoms of anthracnose in red chili	37.56 – 34.18 cm 7.5 – 3.2 fruit 6.2 – 3.8 gram 0.67 – 0.00 cm	(Sari et al., 2020b)
Extract of Curcuma aeruginosa				Mycelium dry weight Symptoms of anthracnose in red chili	80.53 – 0.00 mg 0.88 – 0.00 cm	
Extract of Curcuma zedoaria				Mycelium dry weight Symptoms of anthracnose in red chilies	0.00 mg 0.78 – 0.00 cm	
	Mycelium dry weight	69.00 – 0.00 mg				
Citronella Leaf Extract	50 – 250 g/L	-		When the early symptoms of anthracnose disease appear in red chilies The intensity of the attack to <i>C. capsici</i> Effectiveness and level of fungicidal ability	2.48 – 2.00 days 19 - 20 -11,76 until 17,65%	(Elfina et al., 2016)
Awar-Awar Leaf Extract	1 – 5%	-		Percentage of incidence of anthracnose in red chilies Percentage of incidence of anthracnose in red chilies Disease intensity Yield / amount of red chilies (kg / plant) Losing the salvaged yield of red chilies	41.00 – 0% 41.00 – 0% 38.21 – 0% 0.163 – 0.587 59.51 – 88.76%	(Sudirga, 2016)
Betel and Tobacco Leaf Extract	(1:2), (2:1), (3:1)	(1:2), (1:3),	-	Anthracnose incidence rate	25 – 75%	(Oktarina et al., 2017)
Betel Extract	200 – 600 mL/L	-		The incubation period for anthracnose Number of fruits / plants Number of fruit / plot Fruit weight / plant Fruit weight / plot Number of healthy fruit / plant Number of damaged fruit / plants Percentage of healthy fruit / plot Percentage of damaged fruit / plot The intensity of the plant attacked	4 – 9 days 33 – 36 fruits 75 – 76 fruitd 362.23 – 387.21 gram 757.80 – 777.93 gram 21 – 24 fruits 8 – 5 fruits 88.15 – 97.46% 12.24 – 2.56% 3.06 – 1.25%	(Juniar Dwi Cahya, 2019)
Extract of Putri Malu	30 – 90%	-		Incidence of anthracnose in red chilies Incubation period Spot diameter	62.5 – 0% 6 – 12 days 6.8 – 0 mm	(Eviyanti, 2018)
Betel Leaf Extract	-	-		Incidence of anthracnose in chilies	43 – 15%	(Trisnawati et al., 2019)
Neem Leaf Extract	15% - 45%	-		Average height of chili plants	25.41 – 22.79 cm	(Tobing & Mulyaningsih, 2020)

				Average width of fungal spots	25.02 – 22.80 cm	
				Interaction of average of plant leaf area	33.17 – 33.03 cm <sup>2</sup>	
				Average weight of chilies	5.97 – 4.89 gram	
				Interaction of the main branches of the chili plant	36.67 – 52.22%	
				Dry weight of chilies	20.79 – 15.07 gram	
				Wet weight of chilies	20.53 – 26.74 gram	
				The average of root wet weight	3.58 – 4.99 gram	
				The average of root dry weight	0.36 – 0.49 gram	
				Disease incidence in chilies	20.3 – 0.00%	
				The average of incidence of pest infestation	0.52 – 2.85%	
Banana Weevil Extract	15 - 45%	-		Average height of chili plants	23.57 – 25.49 cm	(Tobing & Mulyaningsih, 2020)
				Average width of fungal spots	24.58 – 23.50 cm	
				Interaction average leaf area of plants	28.99 – 40.21 cm <sup>2</sup>	
				Average weight of chilies	5.42 – 5.83 gram	
				Interaction of the main branches of the chili plant	43.00 – 59.11%	
				Dry weight of chilies	22.17 – 19.63 gram	
				Wet weight of chilies	26.81 – 22.86 gram	
				The average weight of the wet roots	3.98 – 4.44 gram	
				The average dry weight of the roots	0.39 – 0.45 gram	
				Disease incidence in chilies	1.31 – 4.17%	
				The average incidence of pest infestation	2.94 – 1.17%	
Neem Leaf Extract	15 – 45%	-		Height of red chili plant	16.39 – 17.26 cm	(Aziziy et al., 2020)
				Number of red chili leaves	18.47 – 18.89 leaves	
				Number of productive branches	5.11 – 5.33	
				Fruit weight / pPlant	46.95 – 50.03 gram	
				Number of pieces / plant	12.83 – 13.83 fruits	
				Fruit length	10.63 – 10.37 cm	
				Fruit diameter	1.10 – 1.12 cm	
Banana Weevil Mole Extract	15 – 45%	-		Height of red chili plant	16.30 – 17.65 cm	(Aziziy et al., 2020)
				Number of red chili leaves	18.64 – 19.22 leaves	
				Number of productive branches	5.06 – 5.28	
				Fruit weight / plant	44.08 – 53.20 gram	
				Number of pieces / plant	12.19 – 13.89 fruits	
				Fruit length	10.37 – 10.62 cm	
				Fruit diameter	1.11 – 1.12 cm	
Noni Leaf Extract	4 mL/100 mL, 8 mL/100 mL, 12 mL/100 mL, 16 mL/100 mL aquadest	-		Anthracoze intensity	12.75%	(Marsuni, 2020)
Betel and Tobacco Extracts	(1:1), (1:2), (2:1), (1:3), (3:1)	-		Disease incidence rate	75 – 25%	(Nur Rohmah, 2017)
				Incubation period	4 – 9 days	
				Spot diameter	6 – 4 mm	
Kenikir Leaf Extract	5 – 20%	-		Incubation period	4.40 – 5.13 days	(Amelia et al., 2020)
				Disease incidence	72.5 – 52.5%	
				Percentage of incidence of anthracnose in chilies	72.5 – 87.5%	

Tobacco Extract	25 – 100%	-	Disease incidence	75 – 0%	(Isman Duila, 2017)
Extract of Jarak Pagar Seed	30% - 50%	-	Incubation period	4 – 13 days	(Lestari et al., 2020)
			Incubation period	2.57 – 5.4 days	
Extract of Forest Betel Leaf Flour	25 – 100 g/L	-	The incidence of anthracnose in chilies	65 – 85%	(Elfina et al., 2015)
			The appearance of early anthracnose symptoms	3.33 – 4.25 days	
			The intensity of fungal attack on chilies	18.00 – 10.00%	
Soursop, Betel, Papaya, and Garlic Leaf Extracts	-	-	Effectiveness and level of fungicidal ability	14.00 – 52.00%	(Zulkipli et al., 2018)
			The intensity of the attack	39%, 31%, 33%, 35%	
Fragrant Lemongrass Leaf Extract	0.1 – 0.5%	0.2 % b/v synthetic fungicides	Incubation period	5.0 – 6.0 days	(Syabana et al., 2015)
			The intensity of the attack	13.3 – 6.6%	
			Reduction in weight of chilies	13.9 – 7.4%	
			Average total yield	3.8 and 2.5 kg	

Several parameters that are often observed in in vivo tests include: 1) The percentage of incidence of anthracnose disease and 2) The percentage of severity of anthracnose disease or the intensity of disease / attack.

Determination of the percentage of disease occurrences is done by counting the number of symptomatic chilies in each plant with the following formula (Suwastini et al., 2020):

$$TP \frac{n}{N} \times 100\% \quad (4)$$

With :

TP = Occurrence of disease (%)

n = Number of infected (symptomatic) fruit per plant

N = Total number of fruits observed per plant

Anthracnose disease in chilli is characterized by the appearance of blackish brown spots that will expand into soft rot with black dots in the middle which are collection of seta and conidia of *C. capsici* fungi. The attack of *C. capsici* fungi begins by attaching the spores to the fruit and then the spores will germinate. Furthermore, through the fungal hyphae inject the fruit tissue and take nutrients in it so that it can interfere with metabolism and even cause cell death. The more severe the disease attack, the more extensive the rotting area on the fruit will be, this is due to damage to the fruit tissue and even cell death which ultimately results in the fruit experiencing dry rot or shrinking. (Andriyani et al., 2020). Disease severity is the surface area of chilies that shows symptoms of disease. Disease severity can also be interpreted as the part of the plant affected by disease or the disease area of the sample plant. Determination of the percentage of disease severity can be calculated by the formula as follows (Suwastini et al., 2020):

$$TP \frac{\sum nxv}{NxV} \times 100\% \quad (5)$$

With :

KP = Disease severity (%)

N = The number of fruit observed per plant

n = The number of fruits in each attack category

v = Numeric value for each attack category

V = Highest score

Several studies have also identified other parameters in the in vivo test, for example: fruit weight, mycelium dry weight, spot diameter, yield / number of red chilies, number of fruits, effectiveness and level of fungicidal ability, incubation period of anthracnose disease, morphometry of cayenne pepper, period incubation, percentage and fresh weight of healthy cayenne pepper affected by anthracnose disease, when the early symptoms of anthracnose disease appeared in red chilies, and the height of chili plants. The plants studied for chilies had the effectiveness of being used as a natural fungicide. However, many in vivo studies do not compare with synthetic fungicides. So, it is not possible to know the effectiveness of the performance of natural fungicides for chilies when compared to synthetic fungicides. Table 6 shows that suren leaf extract and nut bulbs can be used as alternatives to natural fungicides to help overcome the problem of anthracnose in chilies.

**Table 6** Comparison of the effectiveness of natural fungicides for chilies with synthetic fungicides

Plants	Synthetic Fungicides	Explanation	Reference
Suren Suren Leaf Extract	Mankozeb 1g/L	In general, the performance of suren leaf extract as natural fungicide > mankozeb fungicide	(Andriyani et al., 2020)
Extract of Umbi Teki	Propineb	The treatment of nut bulb flour with concentration of 5%, 15%, and 25% was comparable to the propineb fungicide which is effective in controlling anthracnose in chili plants.	(Sihite et al., 2020)
Fragrant Lemongrass Leaf Extract	0.2% w/v synthetic fungicide	In general, the performance of fragrant lemongrass leaf extract as natural fungicide < 0.2% w / v synthetic fungicide	(Syabana et al., 2015)

## 6 Conclusion

This paper reviews the potential plants as an alternative to chili fungicides, the preparation of test solutions, in vitro and in vivo fungicide tests. The part of the plant that is widely studied as fungicide for chilies is the leaves, while the parts of the plant that are rarely used as samples are the parts of the rhizome, roots, tubers, weevils, seeds, fruit, flowers or all parts of the plant. The methods of extract preparation used as fungicide test include: maceration method, stratified fractionation method, and decoction method. The plants studied had the ability to inhibit the growth of the *Colletotrichum capsici*, *Colletotrichum gloeosporioides*, and *Colletotrichum acutatum*. The 80% alcohol extract and 10% and 60% n-hexane extract of Chinese henna leaves can be equal with the performance of 0.2% propineb by in vitro study. In addition, the 60% and 70% kirinyuh leaf extracts were also able to match acrobat 0.2% performance by in vitro study. Two parameters that are often observed in the in vivo test are the percentage of anthracnose disease incidence and the percentage of anthracnose disease severity. Suren and nut bulbs leaf extract can be used as alternative to natural fungicides to help overcome the problem of anthracnose in chilies.

## Declaration of competing interest

The authors declare no known competing interests that could have influenced the work reported in this paper.

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