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

Anthracnose 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 Men) leaf extract and nut bulbs can be used as an alternative to vegetable/fungal fungicides to help overcome the problem of anthracnose in chilies.

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 chilies which is in line with the growth in population and the development of food industry that require the chilies as raw material (Subagyno 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 chilies 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 chilies (Badan Pusat Statistik Republik Indonesia, 2019).

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Anthracnose 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 capsica fungus attacks ripen chilies that are reddish in color, while Colletotrichum gloeosporioides which has 2 strains, namely: the R strain which only attacks ripe red chilies and the G strain which can attack all parts of the plant, including mature red chilies 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 (Play et al., 2010).

The use of chemical or synthetic pesticides is the most common control. Some examples of synthetic pesticides included: Pyraclostrobin, Azoxystrobin, Picoxystrobin, Difenconazole, 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/fungal 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/fungal 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 (Aglai a odorata L.), mengku/du/moni (Morinda citrifolia L.), mimba/neem (Azadirachta indica A. Juss.), kenikir/compositae (Cosmos caudatus Kunth.), sirih/betel (Piper betle 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 alternative 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 a few researchers have combined 2 plants, for example: mixture of betel and tobacco leaf (Oktarina et al., 2017), Zulkipli et al., 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: Colletotrichum capsici, Colletotrichum gloeosporioides and Colletotrichum acutum. 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              | Cyperus rotundus L.              | Leaves        | In vivo                   | (Shite et al., 2020)             |
| Urang Aring            | Eclipta alba (L.) Hassk           | -             | In vitro                  | (Andreas et al., 2018)           |
| Ketepeng Cena          | Cassia alata Linnaeus            | All parts of plant | In vitro                  | (Arneti & Sulyanti, 2017)      |
| Forest Betel           | Piper aduncum L.                 | Leaves        | In vitro and In vivo      | (Elfina, 2015)                   |
| Fragrant Lemon-grass   | Cymbopogon nardus L.             | Leaves        | In vitro and In vivo      | (Elfina, 2016)                   |
| Tobacco                | Nicotiana tabacum L.             | Leaves        | In vitro                  | (Isman Duila, 2017)              |
| Mixture of Betel and Tobacco | Piper betle L. dan Nicotina tobacum | Leaves      | In vitro and In vivo      | (Oktarina et al., 2017)          |
| Kunyit                 | Curcuma longa sensu Val          | Rhizome       | In vitro and In vivo      | (Sari et al., 2020a)             |
| Temu Putih             | Curcuma zedoaria (Berg.) Roscoe   | -             | In vitro                  |                                  |
| Temu Hitam             | Curcuma aeruginosa Roxb          | -             | In vitro                  |                                  |
| Putri Malu             | Mimosa pudica 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           | Jatropha multifida               | Leaves        | In vivo                   | (Suwastini et al., 2020)        |
| Tembelekan             | Lantana camara H. suaveolens (L.) Poit | Leaves      | In vitro                  | (Chatri & Mansyur-din, 2015)    |
| Karamunting            | Melastoma malabathricum L.       | Leaves        | In vitro and In vivo      | (Suyanti et al., 2020)          |
| Purun Tikus            | Eleoharis dulcis                 | Leaves        | In vitro and In vivo      |                                  |
| Kirinyuh               | Chromolaena odorata L.           |               |                           |                                  |
| Noni                   | Morinda citrifolia               | Leaves        | In vivo                   | (Marsuni, 2020)                 |
| Betel                  | Piper betle L.                   | Leaves        | In vivo                   | (Juniar Dwi Cahya, 2019)        |
| Tagetes                | Tagetes erecta                   | Leaves        | In vitro                  | (Satryawibowo et al., 2015)     |
| Suren                  | Toona sureni Merr.               | Leaves        | In vivo                   | (Andriyani et al., 2020)        |
| Betel                  | Piper betle                      | Leaves        | In vitro and In vivo      | (Andriyani & Purwantisari, 2019) |

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| Plant Name                  | Scientific Name                  | Part(s)                 | Extraction Method(s) | Authors                          |
|----------------------------|----------------------------------|-------------------------|----------------------|----------------------------------|
| Fragrant Lemon-grass       | *Cymbopogon nardus* L.           | Leaves                  | In vitro and In vivo  | Syabana et al., (2015)           |
| Papaya                     | *Carica papaya* Linnaeus         | Leaves                  | In vitro             | Liswarni & Edriwilya, (2020)     |
| Pacar Cina                 | *Aglaia odorata* L.              | Leaves                  | In vitro             | Efri et al., (2017)              |
| Neem                       | *Azadirachta indica* A. Juss.    | Juss                    | Leaves In vivo       | Aziziy et al., (2020)            |
| Kepok Banana               | -                                | Hump                    |                      |                                  |
| Noni                       | *Morinda citrifolia* L.          | Leaves                  | In vitro             | Anggreini et al., (2016)         |
| Neem                       | *Azadirachta indica* A. Juss.    |                         |                      |                                  |
| Kenikir                    | *Cosmos caudatus* Kunth.         | Leaves                  | In vitro and In vivo  | Amelia et al., (2020)            |
| Babadotan                  | *Ageratum conyzoides*            |                         | In vitro             | Wulandari et al., (2015)         |
| Awar-awar                  | *Ficus septica*                  | Leaves                  | In vitro and In vivo  | Sudirga, (2018)                 |
| Mixture of Betel and Tobacco | *Piper betle* L. dan *Nicotiana tobacum* | Leaves | In vitro and In vivo | Anjani, (2018) |
| Gelinggang                 | *Cassia alata* L.                | Leaves                  | In vivo              | Supriati et al., (2016)          |
| Jarak Pagar                | *Jatropha curcas* L.             | Seed                    | In vitro and In vivo  | Lestari et al., (2020)           |
| Mixture of Betel and Tobacco | *Piper betle* L. dan *Nicotiana tabacum* L. | Leaves | In vitro and In vivo | Nur Rohmah, (2017) |
| Binahong                   | *Anredera cordifolia*            | Leaves                  | In vitro and In vivo  | Yulia et al., (2019)             |
| Neem                       | *Azadirachta indica* A. Juss.    | Fruits                  | In vitro and In vivo  | Ali et al., (2012)               |
| Noni                       | *Morinda citrifolia* L.          | Fruits                  | In vitro and In vivo  | Ali et al., (2013)               |
| Betel                      | *Piper betle* L.                 | Leaves                  | In vivo              | Damiri, (2011)                  |
| Noni                       | *M. citrifolia*                  | Fruits                  | In vitro             | Septiana et al., (2013)          |
| Betel                      | *Piper betle* L.                 | Leaves                  | In vivo              | Ningtyas et al., (2013)          |
| Babadotan                  | *Ageratum conyzoides*            |                         |                      |                                  |
| Noni                       | *Morinda citrifolia*             | Leaves, Flowers, and Fruits | In vivo             | Efri, (2010)                     |
| Jarak                      | *Jatropha curcas* L.             | Leaves                  | In vivo              | Wanda et al., (2014)             |
| Mimba                      | *Azadirachta indica*             |                          |                      |                                  |
| Betel                      | *Piper betle* L.                 | Leaves                  | In vivo              | Wati et al., (2014)              |
| Babadotan                  | *Ageratum conyzoides* L.         |                          |                      |                                  |
| Babadotan                  | *A. conyzoides*                  | -                       | In vivo              | Gusmarini et al., (2014)         |
| Siam                       | *C. odorata*                     | -                       |                      |                                  |
| Reed                       | *I. cylindrica*                  | -                       |                      |                                  |
| Teki                       | *C. rotundas*                    | -                       |                      |                                  |
| Camplong                   | *Callophyllum inophyllum*        | Fruits                  | In vitro             | Sholehah, (2012)                 |
| Patchouli Oil              | -                                | -                       | In vitro and In vivo  | Sakerebui & Wahyu, (2013)        |
| Ubhi Ungu                  | *Ipomoea batatas*                | Leaves                  | In vitro and In vivo  | Saputri & Utami, (2020)          |
| Mixture of Kenikir and Betel| *Cosmos caudatus* dan *Piper betle* | Leaves | In vitro             | Maimunah et al., (2019)          |
| Putri Malu                 | *Mimosa pudica*                  | Leaves                  | In vivo              | Eviganti, (2020)                 |
| Kirinyuh                   | *Euphoratorium odoratum* L.      | Leaves                  | In vitro and In vivo  | Indrawati, (2021)                |
| Cinnamon                   | *Cinnamomum burmannii*           | Leaves                  | In vitro             | Darmadi et al., (2021)           |
| Neem                       | *Azadirachta indica*             | Leaves                  | In vitro             | Rahman et al., (2019)            |
| Garlic                     | *Allium sativum*                 | Rhizome                 | In vitro             | Rahman et al., (2019)            |
| Zinger                     | *Zingiber officinale* Rhizome    | Rhizome                 |                      |                                  |
| Termaric                   | *Curcuma longa*                  | Rhizome                 |                      |                                  |
| Tulsii                     | *Osmium sanctum* Linn.           | Leaves                  |                      |                                  |
| Mahogoni                   | *Swietenia mahogoni*             | Leaves                  |                      |                                  |
| Mehendi                    | -                                | -                       |                      |                                  |
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 water bath 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 water bath 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              | 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)            |
| 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 1 x 24 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        | (Evayianti, 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)                     |
| Plant Type                               | Extraction Method | Solvent(s) | Concentration | Notes                                                                 |
|-----------------------------------------|-------------------|------------|---------------|----------------------------------------------------------------------|
| Curcuma spp. Rhizome                    | Maceration        | Methanol   | 1:4           | Concentrated extract (Sari et al., 2020b)                              |
| Urang aring                             | Maceration for 3 x 24 hours then evaporated with rotary evaporator at temperature of 40 °C | Ethanol | 1:1           | Crude extract (Sittisart et al., 2017)                                |
| Shallot and Garlic                      | Extraction and then extracted in centrifuge and filtered | Distilled water | 1:8, 1:10, 1:12 | - (Chávez-Quintal et al., 2011)                                      |
| 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 | -             |                                                                      |
| Noni Leaf                               | Using multilevel extraction | Water, alcohol, acetate 95% ethanol | 1:5          | Dry extract (Putra, 2017)                                             |
| Galangal Rhi-zome, Clove Leaf, and Banggun Bangun | Maceration extraction for 24 hours then evaporated by rotary evaporator | - | -             | Condensed extract (Harianto, 2018)                                   |

### 3 Preparation of extract

The preparation of test solutions for chillies 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 is 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 chillies, 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 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 (Efi 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 Nassau alata Linnaeus sample which was blended with water was carried out for 15 minutes (Arnet 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 vitro tests included: propineb 70%, 0.2% propineb, azoxistrobin, dithiophonamide, 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.
| 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 capsici | 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 (106 spores / mL): 15.8 - 35.2 | (Anjani, 2018) |
| Babadotan Seed | - | Propineb 70% | Colletotrichum capsici | Water extract: 99.28% Methanol extract: 76.81% Ethyl acetate extract: 137.20% N-hexane extract: 85.27% Propineb 70%: 0% | (Wulandari et al., 2015) |
| Noni Leaves and Fruit | 5% | - | Colletotrichum capsici | Percentage of inhibition (%) from 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 capsici | Percentage of inhibition (%): 10.76 – 41.12 | (Amelia et al., 2020) |
| Pacar Cina Leaf | Aquades extract and 10 - 90% alcohol 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 | 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 Percentage of colony diameter: 55.18 - 92.47% Percentage of Spore density: 7.20 - 36.88 | (Andriyani & Purwantisari, 2019) |
| Tagetes Leaf | Water, methanol, ethyl acetate, and n – hexane extracts) | Propineb 70% | Colletotrichum capsici | Percentage of inhibition of C. capsici | (Satryawibowo, 2015) |
| Pasang Surut Weeds | Extract of purun tikus, extract of kirinyuh, and extract of karamunting | - | Azoxystrobin, diphenoconazole, and benomyl | 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 gloeosporioides | Percentage of inhibition (%): 50 - 65 | (Chatri & Mansyur-din, 2015) |
| Putri Malu | 30 – 90% | - | Colletotrichum sp | Percentage of inhibitory power (%): 14.36 - 28.01 The average of spore density (10^6 spores / mL): 19.11 - 4.44 The average of colony growth diameter (cm): 1.50 - 0.58 | (Septianing Ratri, 2017) |
| Curcuma spp | 4 – 12 ppm | - | Colletotrichum capsici | | (Sari et al., 2020b) |

Table 3. In vitro fungicide test on chili test.
| Plant/Extract                                                                 | Application                                                                 | Fungal Strain                          | Percentage of inhibition of fungal colonies (%) | References |
|------------------------------------------------------------------------------|------------------------------------------------------------------------------|----------------------------------------|------------------------------------------------|------------|
| Betel and Tobacco Leaf                                                      | Biorational extract: (1:1), (1:2), (2:1), (1:3), (3:1)                       | Colletotrichum capsici                 | Percentage of inhibition: 0.56 - 0.44          | (Oktarina et al., 2017) |
| Tobacco                                                                     | 25 – 100%                                                                   | Colletotrichum sp                      | Percentage of inhibition (%): 6.56 - 3.78      | (Isman Duila, 2017) |
| Flour of fragrant lemon-grass                                               | 50 – 250 g/l                                                                | Colletotrichum capsici                 | Percentage of inhibitory power (%): 17.47 - 34.43 | (Elfina et al., 2016) |
| Ketepeng                                                                    | 5%                                                                          | Colletotrichum gloeosporioides         | Percentage of inhibitory power (%)             | (Arneti & Sulyanti, 2017) |
| Urang Aring                                                                 | 5 - 25%                                                                     | Antracol                               | Number of mushroom colonies on day 7: 125.75 - 72.75 | (Andreas et al., 2018) |
| Banana and Mimba Leaf                                                       | 15 – 45%                                                                    | Colletotrichum capsici                 | Percentage of inhibition zone for fungal colonies: 10.76 - 6.58 | (Tobing & Mulyaningsih, 2020) |
| Leaf Extract of Ficus septica                                               | 1 – 5%                                                                      | Colletotrichum acutacum               | Colony diameter (mm): 29.72 - 81.39            | (Sudirga et al., 2014) |
| Kirinyuh Leaf Extract                                                       | 10 – 70%                                                                    | Acrobat 0.2%                           | Percentage of spore density (10^6 spores / ml): 63.21 - 99.11 | (Indrawati, 2021) |
| Mansoa alliacea Extract                                                     | 1 – 5%                                                                      | Colletotrichum acutacum               | Colony diameter (mm): 63.25 - 17.00            | (Sudirga et al., 2019) |
| Akar Putri Malu                                                             | 25 - 100%                                                                   | Colletotrichum sp                      | Percentage of inhibition zone diameter (%): 70 - 10 | (Evivanti, 2020) |
| Neem Seed Kernel Extract                                                    | Neem oil, garlic bulb extract, combine application of neem, garlic, ginger, onion plant extract, and neem seed kernel extract (NSKE) | Carbendazim 0.2%                       | 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):

\[ \text{Colony diameter (cm)} = \frac{(D1 + D2)}{2} \]  

With:

- \( D1 \): Vertical diameter
- \( D2 \): Horizontal diameter
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 Riyanto formula (1989) (Gabriel Riyanto, 1989):

\[
C = \frac{t}{nx_{0,25}} \times 10^6
\]  

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 haemacytometer

Colony area was measured using millimeter plotting paper by depicting the colony area on plastic glass (Liswanti 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            | 0.0% 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                                       | (Andres et al., 2018)          |
| Kirinyuh Leaf Extract      | 0.2% Acrobat         | The effectiveness of 60% and 70% kirinyuh leaf extract 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)    |

### 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 anthracnosse        | 8.84 – 8.43%             | (Sitompul, 2017)                   |
| Clove Leaf Putri Malu Root Extract | 30 – 90% | -                   | Incubation period and percentage of disease incidence | 14 – 14.33 days 62.5 – 0% | (Sitompul, 2017)                   |
| Suren Leaf Extract         | 25 – 100%                      | Mankozeb 1g/L       | Spot diameter and morphometry of chili fruit | 91.16 – 99.68% | (Andriyani et al., 2020)          |

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 various 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.
| Extract of Umbi Teki | 5 – 25% | Propineb | Diameter : 1.14 – 1.48 cm
Chili plant height | The severity of anthracnose | 122.8 – 131.6 cm
Plant height | The severity of anthracnose | 4 – 0%
Number of fruit | 13.00 – 17.50%
Fruit weight | (Sihite et al., 2020)
Symptoms of anthracnose in red chili | 37.56 – 34.18 cm
Mycelium dry weight | 7.5 – 3.2 fruit
Symptoms of anthracnose in red chili | 6.2 – 3.8 gram
Mycelium dry weight | 0.67 – 0.00 cm
Symptoms of anthracnose in red chilies | (Sari et al., 2020b)
Number of fruit | 80.53 – 0.00 mg
Fruit weight | 0.88 – 0.00 cm
Yield / amount of red chilies | 0.00 mg
Losing the salvaged yield of red chilies | 0.78 – 0.00 cm

| Extract of Curcuma Longa Sensu | 4 – 12 ppm | - | Mycelium dry weight | 69.00 – 0.00 mg
Symptoms of anthracnose in red chili | When the early symptoms of anthracnose disease appear in red chilies | 2.48 – 2.00 days
Mycelium dry weight | 41.00 – 0%
Symptoms of anthracnose in red chilies | The intensity of the attack to C. capsici | 19 - 20
Mycelium dry weight | Effectiveness and level of fungicidal ability | -11.76 until 17.65%
Symptoms of anthracnose in red chilies | Percentage of incidence of anthracnose in red chilies | 41.00 – 0%
Mycelium dry weight | Percentage of incidence of anthracnose in red chilies | 41.00 – 0%
Symptoms of anthracnose in red chilies | Disease intensity | 38.21 – 0%
Mycelium dry weight | Yield / amount of red chilies (kg / plant) | 0.163 – 0.587
Symptoms of anthracnose in red chilies | Losing the salvaged yield of red chilies | 59.51 – 88.76%
Mycelium dry weight | Anthracnose incidence rate | 25 – 75%
Symptoms of anthracnose in red chilies | (Oktarina et al., 2017)
Mycelium dry weight | The incubation period for anthracnose | 4 – 9 days
Symptoms of anthracnose in red chilies | Number of fruits / plants | 33 – 36 fruits
Mycelium dry weight | Number of fruit / plot | 75 – 76 fruitd
Symptoms of anthracnose in red chilies | Fruit weight / plant | 362.23 – 387.21 gram
Mycelium dry weight | Fruit weight / plot | 757.80 – 777.93 gram
Symptoms of anthracnose in red chilies | Number of healthy fruit / plant | 21 – 24 fruits
Mycelium dry weight | Number of damaged fruit / plants | 8 – 5 fruits
Symptoms of anthracnose in red chilies | Percentage of healthy fruit / plot | 88.15 – 97.46%
Mycelium dry weight | Percentage of damaged fruit / plot | 12.24 – 2.56%
Symptoms of anthracnose in red chilies | The intensity of the plant attacked | 3.06 – 1.25%
Mycelium dry weight | Incidence of anthracnose in red chilies | 62.5 – 0%
Symptoms of anthracnose in red chilies | Incubation period | 6 – 12 days
Mycelium dry weight | Spot diameter | 6.8 – 0 mm
Symptoms of anthracnose in red chilies | Incidence of anthracnose in chilies | 43 – 15%
Mycelium dry weight | The intensity of the plant attacked | (Trisnawati et al., 2019)
Symptoms of anthracnose in red chilies | Incidence of anthracnose in red chilies | (Tobing & Mulyaningsih, 2020)

| Extract of Curcuma zedoaria | 15% - 45% | - | Incidence of anthracnose in red chilies | 30 – 90%
Incidence of anthracnose in red chilies | - | -
Incubation period | Neem Leaf Extract | 200 – 600 mL/L
Number of fruits / plants | 4 – 9 days
Number of fruits / plants | 33 – 36 fruits
Number of fruit / plot | (Juniar Dwi Cahya,
Fruit weight / plant | 2019)
Fruit weight / plot | Number of healthy fruit / plant | 75 – 76 fruitd
Number of healthy fruit / plant | 362.23 – 387.21 gram
Number of healthy fruit / plant | 21 – 24 fruits
Number of healthy fruit / plant | 8 – 5 fruits
Number of damaged fruit / plants | Percentage of healthy fruit / plot | 88.15 – 97.46%
Number of damaged fruit / plants | Percentage of damaged fruit / plot | 12.24 – 2.56%
Number of damaged fruit / plants | The intensity of the plant attacked | 3.06 – 1.25%
Number of damaged fruit / plants | Incidence of anthracnose in red chilies | 62.5 – 0%
Number of damaged fruit / plants | Incubation period | 6 – 12 days
Number of damaged fruit / plants | Spot diameter | 6.8 – 0 mm
Number of damaged fruit / plants | Incidence of anthracnose in chilies | 43 – 15%
Number of damaged fruit / plants | Average height of chili plants | 25.41 – 22.79 cm
Number of damaged fruit / plants | (Tobing & Mulyaningsih, 2020)
| Extract                  | Concentration | Interaction | Average width of fungal spots | Interaction of average of plant leaf area | Average weight of chilies | Interaction of the main branches of the chili plant | Dry weight of chilies | Wet weight of chilies | The average of root wet weight | The average of root dry weight | Disease incidence in chilies | The average of incidence of pest infestation | Height of red chili plant | Number of red chili leaves | Number of productive branches | Fruit weight / pPlant | Number of pieces / plant | Fruit length | Fruit diameter | Height of red chili plant | Number of red chili leaves | Number of productive branches | Fruit weight / plant | Number of pieces / plant | Fruit length | Fruit diameter | Anthracnose intensity | Disease incidence rate | Incubation period | Spot diameter | Incubation period | Disease incidence | Percentage of incidence of anthracnose in chilies |
|--------------------------|---------------|-------------|-------------------------------|------------------------------------------|---------------------------|------------------------------------------------|---------------------|---------------------|-------------------------|----------------------------|-----------------------------|----------------------------------|----------------------|--------------------------|-------------------------|----------------------|------------------------|----------------------|---------------------|-----------------------------|--------------------------|--------------------------|----------------------|---------------------|----------------------|----------------------|----------------|----------------|----------------|----------------|----------------------|------------------------|----------------|----------------|----------------|----------------|---------------------|----------------------|----------------|----------------|----------------|----------------|---------------------|
The percentage of incidence of anthracnose disease can be calculated by the following formula (Suwastini et al., 2020):

\[
TP = \frac{n \times x \times 100}{N \times V}
\]

(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):

\[
KP = \frac{\sum n \times x \times 100}{N \times V}
\]

(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 Leaf Extract | Mankozeb 1g/L | In general, the performance of suren leaf extract as natural fungicide > mankozeb fungicide | (Andriyani et al., 2020) |
| Suren Leaf Extract | 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 chilli plants. | (Shihite 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 Chense henna leaves can be equal with the performance of 0.2% propineb in vitro study. In addition, the 60% and 70% kiriynuh 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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