Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants

Ni Luh Suriani¹, Dewa Ngurah Suprapta¹, Novizar Nazir², Anak. Agung. Ketut Darmadi¹, Ni Made Susun Parwanayoni¹, Ni. Wayan Sudatri¹, Bohari M. Yamin³

Abstract

Rice is a plant that has been intensively bred, especially in Asia, most of the population’s staple food is rice. There were Many types of rice varieties have been developed for high yield production. Different rice diseases arise that many treat to reduce production. Such as curvularia spotting disease caused by the fungus Curvularia sp. Infection of Curvularia species causing leaf spots, leaf blights, kernel root, grain deformation and other diseases are well known globally. It was discovered that the small brown oval spots on the Ciherang rice variety cultivated in Bali, Indonesia was caused by Curvularia verruculosa. This disease could reduce the rice yield up to almost 50%. The application of commercial fungicide by the local farmers has not been satisfactorily successful in term of controlling the disease but instead bring damages to the environment and the soil as well. Farmer’s safety is also another serious issue. The current study develop a natural and green fungicide to check the efficacy of Piper caninum extract against the fungi. Research in the laboratory uses a completely randomized design and research in the field uses a randomized group design. The method of making leaf extracts uses maceration method. Diffusion well technique showed a strong inhibition of the abstract against the C. verruculosa with 22mm inhibition. Zone with a total inhibition of 4% extract concentration. The minimum inhibitory concentration (MIC) was low of 0.5%. Scanning electron images of the treated fungi showed hollow and wrinkled mycelia morphology indicating lysis of the cell has occurred. P. caninum leaf extract at a concentration of 3% provides the highest obstacle to the intensity of C. verruculosa disease, whereas in the treatment of leaf extract concentration p. caninum 4% intensity of C. verruculosa disease has decreased because rice plants are toxic at a concentration of 4%. Further research is needed regarding the treatment of P. caninum leaf extract against C. verruculosa in rice fields.

Key words: Curvularia verrucuosa, Piper caninum, Rice, Scanning electron images.

Introduction

Rice is the main food crop in Indonesia and there are many highly yielding varieties popular in local farmers. Rice production limits on different key factors viz small paddy fields, decreasing soil quality, limited irrigation facilities, as well as disease and pest infestation. One of the emerging rice diseases known as curvularia spotting disease caused by the fungus Curvularia sp. Which is characterized by the curvularia spotting in the form of brown spots on rice plants, especially on the leaves. This disease is also found on Lily plants (Hippeastrum striatum) in China (Yin et al, 2018), in Cymbopogon caesiusdi grass in India (Avinash, 2015). These fungal colonies are brownish black in the middle and grayish black at the edges (Su et al., 2015). Curvularia spots cause the rice spikelets to change color and experience tissue death. Curvularia spotting can cause yield loss of 49.53% in the Ciherang rice species (Karta et al., 2018). Today, farmers deal with Curvularia spotting diseases by using synthetic pesticides (nodox) that have the potential threat to the local agro-ecology. According to Kiss et al. (2020), the curvularia fungus is a pathogen that has a conidia a septa and a hyphae. Curvularia crepinii fungus has reported to have secondary metabolites that are phytotoxic to Echinochloa crusgalli (Yin et al, 2018).

Given the impact of the use of pesticides is very dangerous to the agro-ecology, the local Indonesia government began to launch the organic farming movement ¹Udayana University, Bukit Jimbaran, Badung, Bali, Indonesia. ²Department of Agriculturatal Tecnology, Universitas Andalas, Indonesia. ³Publication Enhancement Unit, Universiti Kebangsaan Malaysia, Bangi, 43600 Selangor, Malaysia. Corresponding Author: Ni Luh Suri, Udayana University, Bukit Jimbaran, Badung, Bali, Indonesia. Email: nilusurianisuriani@yahoo.com How to cite this article: Suri, N.L., Suprapt, D.N., Nazir, N., Darmadi, A.A.K., Parwanayoni, N.M.S., Sudatri, N.W. and Yamin, B.M. (2020), Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants. Indian Journal of Agricultural Research. 54(4): 411-419. (Suprapt, 2014). Organic farming is starting to be in demand by some tourism communities in Bali, such as the development of organic agriculture in Jati Luwih village, Penebel, Tabanan Bali, which is one of the tourist villages (Fumitaka et al, 2015). The increase in use of biopesticides are very appropriate for sustainable agricultural development. These biopesticides can be biological agents derived from plant parts such as plant extracts. Kumari and Shanmugam (2020) in which of the 12 rice varieties tested on the organic farming system in Tamil India obtained culture CB 05022 has the largest production (4.88 tons/ha) can
produce a yield of 32% to 82% compared to the variety that lay. The preliminary results showed that *P. caninum* leaf extract was able to inhibit the *C. verruculosa* fungus which caused curvularia spotting disease in rice plants with a diameter of 22 mm inhibition activity (Preliminary studies, 2019). The *P. caninum* plant is a creeper, living at an altitude of 600-800 m above sea level, humidity of 60%, temperature range of 20-25°C, is a wild plant rarely used by the local farmer communities Suriani et al. (2015; 2019) and Suriani (2016 and 2018) stated that *P. caninum* crude extract was able to inhibit blast disease in rice plants with an inhibitory diameter of 44 mm because it contained substances such as alkaloids, steroids, phenolics and flavonoids. *P. caninum* extract is as anti-bacterial, anti-fungal and as an antioxidant of 77.9% in leaves and 87% in stems (Maj et al., 2004). Extract of some plant like piper has also anti-cancer properties (Tanjug, 2013). Considering all these points, the current study objective was analyse the ability of *P. caninum* extract to inhibit the growth of *C. verruculosa* infecting rice plant in-vitro as well as in controlled environment.

**MATERIALS AND METHODS**

**Plant Materials**

This experiment was carried out in Senganan Village, Penebel District, Tabanan Regency, Bali Province, Indonesia (longitude 115.0; latitude -8.45 and altitude 249 m above sea level). Using red Balinese rice varieties (*Oryza sativa*). The extract used as a treatment was a *P. caninum* extract taken from Senganan Village, Penebel District, Tabanan Regency, Bali Province, Indonesia. This experiment was carried out in the dry season from January to May 2019. Rice seedlings were carried out on January 1, 2019 and rice planting in polybags was carried out on January 20, 2019. Application of *P. caninum* leaf extract was carried out on February 21, 2019. Types of soil used is paddy soil (sandy clay loam) and using compost from cow dung and crop residues, the fertilizer used is organic fertilizer, the size of the bag is 30 cm x 30 cm. Average temperature of 25.9°C with an average humidity of 80%, rainfall on 2178 with full sun exposure. Standard cultivation steps involving seed seeding, planting media preparation, seed planting, plant maintenance and inoculation of pathogenic fungi (*P. oryzae*).

**Piper caninum Extraction Method**

*P. caninum* leaves were collected from Senganan Village, Penebel District, Tabanan Regency (longitude 115.0; latitude -8.45 and elevation 249m above sea level), Bali. The top three matured leaves from branch end were selected and collected. The collected leaves were washed with clean water to remove contaminants, then cut to small pieces and wind dried for 3 days in the shade (Harborne, 1987). The materials (0.5 kg) were then macerated in methanol at a ratio of 1:10 (weight / volume) for 48 hours in the dark, at room temperature. The filtrate was obtained by filtering using 4 layers of gauze followed by filtration using Whatman filter paper No. 1. The filtrate obtained were combined and then evaporated using a rotary evaporator at 40°C to remove the solvent (methanol). The crude extract obtained was ready (Suprapta, 2014).

**Antifungal Crude Extract**

Petri dishes were filled with 200 µl spores of fungal *C. verruculosa* which was then added with 10 ml of an aqueous PDA medium (temperature of about 45°C) was shaken horizontally so that fungal spores and PDA media were evenly mixed. After being condense, as much as two well diffusions were made using cork borer (diameter 5 mm) on each Petri dish. Each well was filled with 20 µl diffusion crude extract of *P. caninum* leaf forest. These cultures were placed in a dark place at a room temperature. Observations were carried out by measuring the diameter of inhibition zone formed around the well diffusion (Astlili et al., 2012).

**Minimum Inhibitory Concentration (MIC)**

The MIC test uses the diffusion well method with concentration of 0%; 0.1%; 0.2%; 0.3%; 0.4%; 0.5%; 0.6%; 0.7%; 0.8%; 0.9%; 1%. Each concentration was repeated 4 times. Observations were made to measure the diameter of the inhibition zone using a ruler (Parwanayoni et al., 2018).

**Test of Inhibitory Activity Extracts Against Fungus Colony Growth**

The concentration of extract inhibitory effect on the growth of *C. verruculosa* colonies was 0% as a control, 1%; 2%; 3% and 4% on PDA media. PDA media were poured into the petri dish and after the media became solid one piece of mycella was taken from the edge of the 5-day-old fungus colony on a PDA using a 7 mm diameter cork was placed in the middle of the Petri dish. This culture was incubated at room temperature in a dark place for several days until the fungus in the control filled the Petri dish. Observations were carried out every day by measuring the fungus diameter in each treatment. The percentage of inhibitory power was calculated by comparing the growth of fungi in the controls treatment and Inhibition was calculated using the formula of

\[
IR (%) = \frac{DC- DT \times 100}{DC}
\]

(Suriani et al., 2015) and Alkooranee et al., 2020).

Where:

- IR=Inhibitory activity against radial growth in percent.
- DC=Diameter of fungus colony without extract treatment (control).
- DT=Diameter of fungus colony treated with extract.

**In-vitro studies of C. verruculosa**

Assessment of the effect of *P. caninum* leaf extract against *P. oryzae* mycella structure was evaluated by mixing 200 µL of the extract concentration of 10% with liquid PDA medium (temperature of about 45°C) in a Petri dish then the Petri dish was shaken horizontally manually. Pieces of fungal colonies taken with a cork borer diameter of 5 mm was placed in the middle of PDA in petri dishes and repeated 3 times. Petri dishes with the mixtures were incubated at 25°C for 3 days. Samples of fungal colony formed in each
Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants

The fungus morphology was examined by using scanning electron microscope energy-dispersive X-ray spectrometry (FE-SEM, ZEISS Merlin operating at 0.2-30kV beam current up to 400nA and the lowest vacuum of few pA-300nA. In the present study 3kV accelerating electron was applied for the imaging and 15kV for EDX experiment except at the husk where 10keV was sufficient (Suriani et al., 2015). This analysis was carried out at the Chemistry laboratory, The National University of Malaysia.

Statistical Analysis

Laboratory experiments for the application of Piper caninum leaf extract to C. verruculosa, using a completely randomized design, consisting of 5 treatments and 4 replications (F0 = control without extract; F1 = extract 1%; F2 = extract 2%; F3 = extract 3%; F4 = extract 4%). Experiment in greenhouse based on Group Random Design 5 treatments and 4 replications. Altogether there are 20 experimental units and each unit consists of 5 pot. Each pot took only 2 seeds. Each treatment labeled as F0 for control; F1 = treatment with (10% compost + extract 1% mixture); F2 = treatment with (10% compost + 2% extract mixture); F3 = treatment (10% compost + extract 3% mixture) and F4 = treatment with (10% compost + 4% extract mixture). One-way Analysis of Variance (ANOVA) methods using SPSS program online.

RESULTS AND DISCUSSION

Inhibitory Activity of Crude Extract

Fungus C. verruculosa has brownish black colonies in the middle and greyish black at the edges (Fig 1). Under the microscope at 10X40 magnification the greyish black mycelia of the isolated fungus was be observed (Fig 2).

P. caninum crude extract showed inhibitory on the fungus verruculosa with a diameter of inhibitory zone of strong category of 22 mm diameter (Fig 3). The P. caninum extract has anti-bacterial, antifungal and antioxidants properties due to the present of alkaloid phytochemicals, flavonoids, polyphenols and steroids (Suriani, 2016) and an active compound tetradecane, octadecamethyl cyclononasiloxane and benzenedicarboxylic acid which is an anti-fungal.

In previous study, P. caninum crude extract displayed strong activity against Pyricularia oryzae (Suriani et al., 2015). Similar result was also observed in the present study against C. verruculosa as shown in Fig 3. The black spot in the middle is the extract and the white fungi grows to certain limit around the extract creating an inhibition zone. At 4% extract concentration the diameter of the inhibition zone was 22 mm.

It has been reported that the P. caninum extract contains alkaloid, flavonoids, steroids and polyphenols. Some individually identified chemical components are N-isobutyl-15-(3’,4’-methyleneoxyphenyl)-2E,4E-12E-pentadecatrienamide, N-isobutyl-(2E,4E,14Z)-eicosatrienamide and six flavonoids; 5-hydroxy-7-methoxyflavone, 4’-hydroxy-5,7-dimethoxyflavone, 4’5,7-trimethoxyflavone, 5,7-dimethoxyflavone, 2’-hydroxy-4’6’-dimethoxychalcone and 5,7-dimethoxyflavanone. The essential oils in the leaves are safrrole, β-caryophyllene, β-pinene and germacrene and linalool have also been reported in previous studies. These essential oils also possess strong antibacterial and antifungal activities (Salleh et al., 2011, 2015; Suriani, 2016; Appuaka, 2013; Chouhan et al., 2017; Mandrianariveloa, 2009).

Minimum Inhibitory Concentration (MIC)

The knowledge of minimum inhibitory concentration of P. caninum leaf extract is important in the management of pesticide application. The MIC value is the minimum concentration to see the Efficacy of the biocidal agent. Low concentration is certainly preferred because excessive amount of extract may become toxic to the plant and could

Fig 1: C. verruculosa colony.

Fig 2: C. verruculosa colonies 10 x 40.

Fig 3: Inhibitory activity of P. caninum extract against C. verruculosa.
Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants

damage the wax layer on the leaves affecting the efficiency of photosynthesis process and other non-target organisms. The MIC value for the extract against *C. verruculosa* fungus was found to be 0.5% similar to keben (*Barringtonia asiatica*) seed extract against the same. On the other hand, the MIC value of the flavonoid from the crude extract of *P. caninum* against *Escherchia coli, Staphylococcus aureus, Bacillus substilis, Psedomonas areginosa* and *P. putida* bacteria was 125-1000 μg / mL and 500 μg / mL against *Aspergillus niger* (Salleh, 2015; 2011). Another important information in the formulation of fungicide for field application is the concentration of the agent that can Control the pathogen. Learned by inhibition of fungal colonies by varying the concentration of *P. caninum* crude extract at a concentration of 0.5% (the smallest concentration) which can inhibit *C. verruculosa* causing brown spots. Inhibition below the concentration of 5% or the same is an effective concentration for biopesticides and is worthy of being recommended for use as biopesticides. The lower the MIC concentration of an extract, the more effective the extract can inhibit a pathogen and the more efficient it is in using a plant resource. The use of high concentrations also causes toxic plants because the phytochemical substances in the extract will damage the wax layer on the leaves of the plant, so the leaves will wither and not be able to photosynthesis (Suprapta, 2014). In previous studies, flavonoid compounds from *P. caninum* extract have MIC of 125 µg / mL against *Escherchia coli, Staphylococcus aureus, Bacillus substilis, Psedomonas areginosa* and *P. putida* (Salleh, 2015; 2011), *Hyleocerius undatus* leaf crude extract with MIC 12 mg / ml against *Fusarium oxysporum f. sp. capsici* causes of wilt in parika (Suprapta and Khalimi, 2012). The high alkaloid content of *P. caninum* extract is antibacterial, has a MIC of 125 µg / mL against *Bacillus substilis* (Wan et al., 2015). Ekstrak Acacia tortilis subsp dapat menekan penyakit Fusarium oxysporum f.sp. albedinis, the cause of Bayoud Disease of the date palm in Southwest Algeria dengan MIC 3 µg/ml (Mezouari et al, 2019).

Inhibitory test on fungus colonies was carried out to calculate the inhibitory activity of extracts against the pathogen. The inhibition of 1% and 2% extract concentration on the fifteenth days was 27.8% and 35.6%, respectively, which is medium inhibitory (F 1 and F2, Fig 4) The inhibition 54% was displayed by 3% extract concentration (Fig 4, F3). A total inhibition was observed at 4% concentration as shown in Fig 4 F4. One way to calculate the inhibitory activity of extracts against pathogenic fungus is to use the inhibitory test on fungus colonies. At extract concentrations of 3% and 4% classified as very strong inhibitory activity because of more than 75% (Fig 4). All extract concentrations tested were significantly different from controls on the fifteenth days (P <0.05) and thus were also significantly different between treatments (Table 1 and Table 2). All extract concentrations tested under the current study were significantly different against controls (Fig 4). The inhibitory values are shown in Table 1. Good correlation between concentration and inhibition zone parameters was observed (Fig 5). The extract treatment of 1% and 2% on the 15th days was able to inhibited fungal *C. verruculosa* by 27.8% and 35.6% which is classified as medium inhibitory. 3% extract concentration was able to inhibit 54.4% classified as strong inhibition and 4% extract concentration could inhibit 100% (Table 1) classified as very strong inhibition.

![Fig 4: Inhibitory Activity of P. cajinum leaf Extract on C. verruculosa the causal agent of C. spooting disease on rice. It is good to note down here the concentration for F above.](image_url)
Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants

The inhibition of *P. caninum* against the fungal colonies of *Pyricularia oryzae* ranged from 11.83-37% at concentrations of 0.5-3.5% on PDA media (Suriani *et al.*, 2015). Higher concentration of *P. caninum* extract resulted in higher inhibition of *C. verruculosa* fungus (Fig 5). This is because higher extract concentration contain higher amount of phytochemical content which functions as an antimicrobial (Nadri *et al.*, 2015). Nasrine *et al* (2017) stated that the water plant extract of *Artemisia herb alba* can inhibit 40% growth of *Fusarium sp* fungal colonies effectively at concentrations 20%. Similarly, bioactive substances in crude extract of *cinnamon* leaf can inhibit fusarium fungal colonies causing stem rot disease in tomatoes and dragon fruit (*Darmadi et al.*, 2016; 2017).

**Effect of Extract on Fungal Mycelia**

The effect of plant extract on the mycelia of *C. verruculosa* was observed using scanning electron microscope. Fungus in control treatment showed a clear undamaged mycelia as shown in Fig 6B however Fig 6A shows the mycelia of the treated fungus was damaged and appeared shrink and hollow. Such morphological changes indicate the lysis of the fungus had occurred. The observation of fungus mycelia of *C. verruculosa* between control and treatment, using SEM (Scanning Electron microscope). There appeared to be a difference between mycelia control and treatment.

Phytochemical substances include alkaloids, flavonoids, polyphenols and steroids found in extracts *P. caninum* can lyse mycelia of *C. verruculosa* as indicated by small and hollow mycelia. This is because alkaloids can damage the permeability of bacterial and fungal cell walls whilephenol can damage proteins in fungal and bacteria cell walls causing the contents of the cell will go out resulting in lysis (Upadhyay, 2014). *Atuna racemosa* Raf. extract contains phytochemicals glycosides, tannins, phenolic and saponins compounds that function to inhibit *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enterica* ser. *Typhimurium*, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans* on agar media by the diffusion well method (*Gentallan et al.*, 2019). Suriani *et al.* (2015) also stated that *P. caninum* extract can lyse the fungi mycelia of *Pyricularia oryzae* causing blast disease in rice, seen by using SEM. Crude extract of cinnamon leaf can lyse fusarium fungi that cause wilt on tomato plants, seen using SEM (*Darmadi et al.*, 2015). Crude extract from *Epicoccum sp* leaf can inhibit the formation of apressoria in *P. oryzae* fungal seen using SEM (*Sena et al.*, 2013). Susun *et al.* (2018) states that *Alamanda cathartica* leaf extract in combination with leaf extract *Mansoa alliace* can lyse the fungal *Athelia rolfsii* causes stem rot disease in peanut plants. Rasu and Hossain

---

**Fig 5:** Relationship between extract concentration with diameter colonies of *C. Verruculosa*.

**Fig 6A:** SEM Mycelia of *C. verruculosa*. Treatment 4%.

**Fig 6B:** Control (1000X Magnification).

**Fig 7:** Intensity development of Curvularia disease.
Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants

(2015) stated that Allamanda cathartica extract can inhibit fungal Curvularia lunata causing curvularia spotting disease in rice.

**Intensity of curvularia disease**

The results showed that *P. caninum* leaf extract was highly influential on the intensity of curvularia in Bali red rice. The 3% extract concentration provides the highest obstacle to the intensity of curvularia disease. Table 2 through 6 show that the treatment and control were significantly different. Controls from week 2 to week 6 showed an increase in the intensity of curvularia spotting with 79.12% in week 8 and continued to increase over time to 82.79% at week 12.

According to Kusai *et al.* (2015) that Curvularia sp is a plant pathogen, especially attacking grasses, cereals and also rice plants, resulting in sharply reduced rice yields. Whereas in the treatment with *P. caninum* leaf extract the intensity of curvularia disease decreased to an extract concentration of 3%, while the concentration of the extract 4% increase in disease intensity, this is also shown in Fig 7. This proves extracts in excessive concentrations will have toxic effects on plants, because rice plants undergo lysis so that the defense of rice plants decreases and is easily attacked by curvularia. *P. caninum* leaf extract can suppress the pathogenic curvularia, although the pathogenic curvularia also has phytotoxic substances against other organisms such as inhibition of bacteria such as Eschericia coli and also has antioxidant activity and inhibition of acetylcholinesterase (Kaaniche *et al.*, 2019). This is also supported by research by Oraon and Mondal (2020) that the initial growth of *Cicer Arietinum* L. was hampered after being given a Putranjiva roxburghii Wall leaf extract license. at high concentrations (100%), because the extract contains allelopathic substances.

Besides the phytochemical substances in curvulria fungus can also inhibit the fungus Phytophthora capsici pathogens in vegetables (Mondol *et al.*, 2017). Although curvularia fungus has phytotoxic properties, *P. caninum* leaf extract can strongly inhibit the development of curvularia fungus. According to Tanin and Soytong (2017), in Cambodia curvularia spots caused by Curvularia lunata can be reduced by using the biological agent Chaetomium cupreum. According to Kithn and Daiho (2014) that the Millettia pachyacapa root extract can inhibit 55.78% at a concentration of 10% and the extract of Acorus calamus can inhibit 53.40% at a concentration of 10% against the curvularia lunata spotting disease and the biological agent Trichoderma harzianum can inhibit 68.85%. Research of Mohana *et al.* (2011) in southern India extracts of Acacia nilotica, Caesalpinia coriaria, Decalepis hamiltonii, Emblica officinalis, Lawsonia inermis and Mimusops elengi can inhibit several rice diseases including curvularia spotting at concentrations of 3500 μg / ml with inhibitory activity ranging from 62.5%-78%.

**Table 1:** Inhibitory activity *P. caninum* leaf extract on *C. verruculosa* colonies on the fifteenth Days.

| Treatment | Diameter colonies | Inhibitory activity to control (%) |
|-----------|-------------------|-----------------------------------|
| F0        | 90a*              | -                                 |
| F1        | 65b               | 27.8                              |
| F2        | 58bc              | 35.6                              |
| F3        | 41c               | 54.4                              |
| F4        | 0d                | 100                               |

* Values followed by the same letters at the same column show no significant different based on Duncan’s Multiple range Test at the P = 5% or P > 0.05.

**Table 2:** Effect of *P. caninum* Extract on Intensity of Curvularia disease and inhibition activity after 8 weeks.

| No  | Treatment | Intensity of Curvularia disease (%) | Inhibition activity (%) |
|-----|-----------|------------------------------------|-------------------------|
| 1   | F0        | 79.12a*                            | -                       |
| 2   | F1        | 51.13b                             | 35.77                   |
| 3   | F2        | 32.15c                             | 60.12                   |
| 4   | F3        | 8.21e                              | 90.77                   |
| 5   | F4        | 8.29e                              | 89.39                   |

* Values followed by the same letters at the same column show no significant different based on Duncan’s Multiple range Test at the P = 5% or P > 0.05.

**Table 3:** Effect of *P. caninum* Extract on Intensity of Curvularia disease and inhibition activity after 9 weeks.

| No  | Treatment | Intensity of Curvularia disease (%) | Inhibition activity (%) |
|-----|-----------|------------------------------------|-------------------------|
| 1   | F0        | 80.01a*                            | -                       |
| 2   | F1        | 48.12b                             | 39.89                   |
| 3   | F2        | 30.17c                             | 62.29                   |
| 4   | F3        | 6.91e                              | 91.36                   |
| 5   | F4        | 7.13e                              | 91.09                   |

* Values followed by the same letters at the same column show no significant different based on Duncan’s Multiple range Test at the P = 5% or P > 0.05.

**Table 4:** Effect of *P. caninum* Extract on Intensity of Curvularia disease and inhibition activity after 10 weeks.

| No  | Treatment | Intensity of Curvularia disease (%) | Inhibition activity (%) |
|-----|-----------|------------------------------------|-------------------------|
| 1   | F0        | 81.21a*                            | -                       |
| 2   | F1        | 47.17b                             | 41.92                   |
| 3   | F2        | 29.55c                             | 63.61                   |
| 4   | F3        | 6.41e                              | 92.11                   |
| 5   | F4        | 6.93e                              | 91.47                   |

* Values followed by the same letters at the same column show no significant different based on Duncan’s Multiple range Test at the P = 5% or P > 0.05.
Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants

Table 5: Effect of P. caninum Extract on Intensity of Curvularia disease and inhibition activity after 11 weeks.

| No | Treatment | Intensity of Curvularia disease (%) | Inhibition activity (%) |
|----|-----------|------------------------------------|------------------------|
| 1  | F0        | 82.31a*                            | -                      |
| 2  | F1        | 46.01b                             | 44.10                  |
| 3  | F2        | 28.92c                             | 64.87                  |
| 4  | F3        | 6.21e                              | 92.46                  |
| 5  | F4        | 6.67e                              | 91.90                  |

* Values followed by the same letters at the same column show no significant different based on Duncan’s Multiple range Test at the P = 5% or P > 0.05.

Table 6: Effect of P. caninum Extract on Intensity of Curvularia disease and inhibition activity after 12 weeks.

| No | Treatment | Intensity of Curvularia disease (%) | Inhibition activity (%) |
|----|-----------|------------------------------------|------------------------|
| 1  | F0        | 82.79a*                            | -                      |
| 2  | F1        | 45.89b                             | 66.00                  |
| 3  | F2        | 28.15c                             | 65.23                  |
| 4  | F3        | 6.02e                              | 92.73                  |
| 5  | F4        | 6.50e                              | 92.15                  |

* Values followed by the same letters at the same column show no significant different based on Duncan’s Multiple range Test at the P = 5% or P > 0.05.

CONCLUSION
The current study concluding that the crude extract of P. caninum leaf can inhibit the growth of C. verruculosa fungal colony, with a 0.5% MIC. Through SEM observation it was showed that the fungal cell wall with the extract treatment becomes smaller, shrinks and cell wall leaks occur. The main outcome of the current study was that P. caninum leaf extract at a concentration of 3% gives the highest level of competence in curvularia disease cause by C. verruculosa. In future there is urgency to research on the application of the extract in open rice cultivated field to determine the in-situ inhibitory activity on Against pathogen.

ACKNOWLEDGEMENT
The authors would like to thank to LPPM Udayana University for funding this research through Udayana University INNOVATION research and also to the Udayana University biopesticide laboratory that has facilitated this research. Special thanks to Research and Instrumentation Centre of Universiti Kebangsaan Malaysia for the morphology and elemental distribution analysis.

REFERENCES
Alkooranee, J.T. Al-khshemawee, H.G. badri, M.A.K.L. Al-srai, M.S. Dawer, H.H. (2020). Antifungal activity and GC-MS detection of leaves and roots parts of Chenopodium album extract against some phytopathogenic fungi. Indian Journal of Agricultural Research. 54(1): 117-121.

Almaguer, M., A. Balista and V.D. Amador. (2012). Effect of Temperature on Growth and Germination of Conidia in Curvularia and Bipolaris Species Isolated from the Air. Aerobiologia. Springer Science: London.

Appuaka, A., Ekwenchi, M.M., Adashak, D.A. and Dildar (2013). Biological Activities of 537 Characterized Isolates of H-Hexane Extract of Azadirachta indica A. Jus (Neem) Leaves. J. Nature and 538 Science. 11(5):141-147.

Asstiti, N.P.A. and Suprarta, D.N. (2012). Antifungal Activity of Teak (Tectona grandis L.F.) Leaf Extract Against Arthrinium Phaeospermum (Corda) M.B. Ellis. The Cause of Wood Decay on Albizia Falcata (L.) Fosberg. J.ISSAAS. 18(1): 62-69.

Chouhan, S.K. Sharma and S. Guleria, (2017). Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines. 4(58): 1-20.doi:10.3390/medicines 4030058:

Darmadi. A.A.K, Suprarta, DN, Gianastra, I.K. (2016). Leaf extract Formula Cinnamomum burmanni Blume on of Fussarium oxysporrum wilt that Attacks Tomato Plants in Bali. International Journal Pure and Applied Bioscience. 4(4): 33-38.

Darmadi. A.A.K, Suprarta, DN, Temaja, R.M.T, Swantara, I.M.D. (2015). Leaf Extract of Cinnamomum burmanni Blume Effectively suppress the growth of Fussarium oxysporrum wilt disease on tomato. Journal of Biologi Agriculture and Healthcare. 5(4):131-137.

Darmadi. A.A.K, Suprarta, DN, Temaja, R.M.T, Swantara, I.M.D. (2017). Gc-Ms Analysis of Active Compound of Cinnamomum Leaf Extract. International Journal of Pharma and Bio Sciences. 9(1): 114-118.

Fumitaka, S. Nobuio, S. Naumi, A. Suprarta, D.N, Nurwulan, A. Youji, N and Masakazu, K. (2015). Kination and Dissemination of Organic Rice Cultivation in bali, Indonesia. Sustainability. 7(5): 5171-5181.

Gentallan Jr. Altoveros, N.C. Borromeo, T.H and. Macabocha, C.G.A. (2019). Antimicrobial and Phytochemical Properties of Atuna racemosa Raf. Kernel Extract. Indian J. Agric. Res. 53(6): 733-736.

Harborne, J.B. (1987). Metode Fitokimia. Penuntun Cara Modern Menganalisis Tumbuhan (terjemahan). Padmawinata, K., Soediro, I., penerjemah. Bandung: ITB.

Hidayat, J, Margaret, A.L. Hanna, Y and Lies K. W. (2013). Isolates of H-Hexane Extract of Azadirachta indica A. Jus (Neem) Leaves. J. Nature and Science. 11(5):141-147.

Kaaniche, F. Abdelaaty, A. Ahmed, S. Abdel, R, Daniel, W. Negeri, A. Imené, Z.E.I, Noureddine Allouche, Lotfi Melloul, Mohamed Mashaan, Norbert Sewald. (2015). Bioactive secondary metabolites from new endophytic fungus Curvularia. sp isolated from Rauwolfia macrophylla. PLOS ONE. 6 (27): 1-12. | https://doi.org/10.1371/journal. pone.0217627.

Karta, S.W. Suprarta, D.N, Khalmi, K. (2018). Uji Aktivitas Antijamur Ekstrak Biji Keben [Barringtonia 579 asiatica (L.) Kurz] terhadap Curvularia verruculosa Penyebab Penyakit Bercak Curvularia pada Tanaman 580 Padi [Oryza sativa L.]. Journal of Tropical Agroecotechnology. 7(3): 412-427.

Kawuri, R. (2012). Pemanfaatan Streptomycyes thermocarboxyldus untuk Mengendalikan Penyebab Penyakit Busuk Daun...
Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants

pada Lidah Buaya (Aloë barbadensis Mill.) Di Bali (desertas). Denpasar: Universitas Udayana.

Kithan, C and Dahoi L. (2014). In Vitro Evaluation of Botanicals, Bio-Agents and Fungicides against Leaf Blight of Eltingera linguliformis Caused by Curvularia lunata Var. Aeria. J Plant Pathol Microbiol An open access Journal. 5(3): 1-6.

Kumar, S, A.A Lal, N. Kumar, S.Jaiswal, H. Kumar, A. and Kumar, M. (2017). Effect of bio control agents and botanicals against Blast of Paddy caused by Pyricularia oryzae. International Journal of Chemical Studies. 5(1): 314-318. 2017.

Kusai, N.A. Madihah, M.Z.A. Shahrizim, Z. ohd, O.T. Nur, A.I.M.Z. (2015). Morphological and molecular characterization of Curvularia and related species associated with leaf spot disease of rice in Peninsular Malaysia. Rendiconti Lincei. Scienze Fisiche e Naturali. 4(9): 1-10.

Law W-F, H.L. Hooi-Leng Ser, T.M. Khan, L-H. Chuah, P. Pusparaja, K-G. Chan, B-H. Goh and L-H. Lee. (2017). The Potential of Streptomycyes as Biocontrol Agents against the Rice Blast Fungus, Magnaportheoryzae (Pyricularia oryzae). Front Microbiol. 8(3). 10.3389/fmicb.2017.00003.

Maj, Jones, S.H., Marshall, R., Johnson, R.K. and Hecht, S.M. (2004). A DNA-damaging oxoporphine alkaloid from Piper caninum. Departments of Chemistry and Biology. University of Virginia. USA.

Mezouari, A. Makhlioui, A. Bendjima, K. Benlirbi, L. Boulanouar, A. Makhliou, K and Jesus Gonzalez, M.D. (2019). Antifungal activity of Acacia tortilis subsp. raddiana tar on Fusarium oxysporum f.sp. albedinis, the cause of Bayoude Disease of the date palm in Southwest Algeria. Indian J. Agric. Res. 53(6): 713-717.

Mohana, D.C. Praveen, P. Veena, V. Koteshwara A.R. (2011). Plant Extract Effect on Seed-Borne Pathogenic Fungi from Seeds of Paddy Grown in Southern India. Journal of Plant Protection Research. 51(2).

Mondol, M.A.M. Jannatul, F. Mohammad, T.I. Anja, S. and Hartmu, L. (2017). Metabolites from the Endophytic Fungus Curvularia sp. M12 Act as Motility Inhibitors against Phytophthora capsici Zosspores. J. Nat. Prod. 80: 347-355. DOI:10.1021/acs.jnatprod.6b00785.

Noemi, K. Mónika, H. Palanisamy, M. Arumugam, M. Kristzina, K. Rajaraman, R. Mónika, V. Tamás, P. Csaba, V. László, K. and Sándor, K. (2020). New Species of the Genus Curvularia. C. tamlindaeunisus and C. coibatorenisis from Fungal Keratitis Cases in South India. Pathogens. 9(9): 1-14. doi: 10.3390/pathogens9010009.

Oraon, S. Mondal, S.(2020). Studies on Allelopathic Effect of Aqueous Leaf Extract of Putranjiva Roxburghii Wall. on Seed Germination and Early Growth of Chickpea (Cicer Arietinum L.). Indian Journal of Agricultural Research. 54(2): 193-198.0.

Parwanayoni, NMS., Supraptad, D.N., Temaja., IGRM, Swantara, IMD., Khalimi, K. (2018). Synergistic Activity 608 of Leaves Extracts of Mansoa alliacea L. and Allamanda cathartica L. to Inhibit Athelia rolfsii, the Cause 609 of Stem Rot Disease in Peanut Plants. Biology Agriculture and Healthcare 8(4): 29-35.

Rajat, G. Katon, D. Payel, N. Panchali. D. (2014). An Overview piper Species for their Biological Activities. International Journal of Pharma Research. 3(1):67-75.

Randrianariveloa, R.S. Sarterb, E. Odoux, P. Bratc, M. Lebrunc, B. Romestandd, C. Menute, H.S. Andrianoselisof, M. Raherimandimbiny and P. Danthuuh. (2009). Composition and antimicrobial activity of essential oils of cinnamomo fragrans. Food Chemis. 1(14): 680-684.

Razu, M.A.U. and I. Hossain. (2015). Eco-friendly management of rice diseases. International Journal Appl. Sci Biotechnol. 3(1): 80-88.

Salleh, W.M.N.H.W., Ahmd, F., Yen. K.H. (2011). Chemical Compositions, Antioxidant and Antimicrobial Activities of Essential Oils of Piper caninum Blume. Int J Mol Sci. 12 (11): 7720-7731.

Salleh, W.M.N.H.W., Ahmd, F., Yen. K.H. (2015). Chemical Constituents from Piper caninum Antibacterial Activity. Journal of Applied Pharmaceutical Science. 5(06): 20-25.

Sena, A.P.A., Amanda, A., Chaiub; Marcio, V.C.B., Cortes., Siva, G.B., Loho, V. L., Prabu, A.S., Filippí, M.C.C.; Arauji, L.G. (2013). Increased Enzymatic Activity in Rice Leaf Blast Suppression by Crude Extract of Epicoccum sp. International Journal of Trop. Plant Pathol. 38(5): 23-42.

Supraptad, D.N and Khalimi, K. (2012). Anti-fungal Activities of Selected Tropical Plants from Bali Island. J. Phytopharmacology. 2(2): 265-270.

Supraptad, D.N. PestsidaNabati Potensi dan Prospek Pengembangan. (2014). Pelawa Sari. Denpasar.

Suriadi, N. L. (2019). Piper caninum blume leaf extract and compost to suppress blast disease and increase the production of bali red rice (Oryza sativa) in green house. International Research Journal of Engineering, IT and Scientific Research. 5(4): 46-54. https://doi.org/10.21744/irjeis.v5n4.693.

Suriadi, N.L. (2019). Utilization of piper caninum blume leaf extract combined with compost to suppress blast disease and increase the growth of local rice Bali (Oryza sativa) in Vivo. International Journal of Life Sciences. 3(2): 33-40. https://doi.org/10.29332/ijls.v3n2.305.

Suriadi, N.L., Darmadi, A.A.K. Parwanayoni N.M.S. (2019). Hamid. The combination of Piper caninum Blume Leaf Extract and Compost Fertilizer for Pressing Blast Disease and Improving Growth of bali Red Rice (Oryzasaativa Linn). IJASEIT Journal. (2): 518-525.

Suriadi, N.L. Darmadi, A.A.K. Supraptad, D.N. Khalimi, K. and Parwanayoni, N.M.S. (2019). The Combination of Piper Caninum Blume and Piper Betel Var. Nigra Leaf Extract to Press Blast Disease on Oryza Sativa Linn in Vivo. The Journal of Research on the Lepidoptera. 50(4): 196-204.

Suriadi, N.L. Darmadi, A.A.K. Supraptad, D.N. Khalimi, K. and Parwanayoni, N.M.S Nazir, N. Ahma, Z.B.Z. Bohari, M.Y. (2019). Antagonism trichoderma sp for pressing blast disease on red bali rice plants (oryza sativa). Journal of Adv Research in Dynamical and Control Systems. 11(10): 70-76.

Suriadi, N.L., D.N.Supraptad, I.M. Sudana.I. M and R. M. Temaja, (2015). Antifungal activity of Piper caninum against Pyricularia oryzae Cav. The cause of rice blast disease on rice. International Journal ISTE. 5(8):72-78.

Suriadi, N.L. (2016). Identification of the Substance Bioactive Leaf Extract Piper caninum Potential as Botanical Pesticides. International Journal IJPAB. 4(4):26-32.
Inhibitory Activity of Piper Caninum Leaf Extract against Curvularia Spotting Disease on Rice Plants

Suriani, N.L. (2018). Bioactive Substance use of Leaf Extract of *Piper caninum* Blume Pressing for Blast Disease and Increase Production in Rice. International Journal IJLS. 2(2): 42-50.

Susun, N.M.P, Suprapti, D.N, Temaja, R.M.T, Swantara, I.M.D, Khamdan, K. (2018). Synergistic Activity of Leaves Extracts of Mansoa alliacea L. and Allamanda cathartica L. to Inhibit Athelia rolfsii, the Cause of Stem Rot Disease in Peanut Plants. Journal of Biologi Agriculture and Healthcare. 8(4): 29-35.

Tanjung, W.A.S. (2013). Obat Tradisional dan Metabolit Sekunder. Fakultas Biologi UGM. Yogyakarta.

Tann, H and Soytong, K. (2017). Biological Control of Brown Leaf Spot Disease Caused by Curvularia lunata and Field Application Method on Rice Variety IR66 in Cambodia. AGRIVITA Journal of Agricultural Science. 39(1): 111-117.

Upadhyay, A., Upadhyaya, I. Kollanoor-Johny, A and Venkitanarayanan, K. (2014). Combating pathogenic microorganisms using plant-derived antimicrobials. Hindawi Publishing Corporation BioMed Research International. 1-12. ID 761741 DOI: 10.1155 / 2014/761741.

Vasantha Kumari, K. Shanmugam, P.M. (2020). Evaluation of Rice (*Oryza Sativa*) Varieties Suitable for Organic Farming. Indian Journal of Agricultural Research. 54(1): 71-76.

Wan, M.N.H.W.S, Farediah. A, Khong, H.Y. Chemical constituents from *Piper caninum* and Antibacterial Activity. (2015). Journal of Applied Pharmaceutical Science. 5(06):20-36.

Yin, C. Liping, J. Feifei, S.Xiao, X. Mingwei, S. and Ying, I. (2018). Phytotoxic and Antifungal Metabolites from Curvularia crepinii QTYC-1 Isolated from the Gut of Pantala flavescens. Molecules. 23(051): 1-9. doi: 10.3390/molecules23040951.

Yin, L, Shuang, F.R, Jayarama, B, Kevin, D.H, Yong, W, De, G.Z. (2018). *Curvularia microspora* sp. Nov. Associated with Leaf Diseases of Hippeastrum Striatum in china. Journal Mycokeys. 29: 49-61.

Yue, HW, Susan, L, Morris,N, Jun, Y, Hong, M.N, Chun, L.L, Kuo. H.L. (2014). Anticancer principles from Medicinal Piper Plants. Journal of Traditional and Complementary Medicinal. 4(1): 8-16.