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THE POTENCY OF CALABUR TREE (*Muntingia calabura*) LEAF EXTRACT TO CONTROL ANTHRACNOSE OF PAPAYA FRUIT

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ABSTRACT

The potency of calabur tree* (Muntingia calabura) *leaf extract to control anthracnose of papaya fruit. The papaya anthracnose caused by *Colletotrichum gloeosporioides* is one of the factors causing a decrease in papaya production. The research aimed to determine the abilty of calabur tree leaf extract in inhibiting growth, sporulation, and viability of *C. gloeosporioides* as well as incubation period and disease severity on the fruit of papaya. The experiment was arranged in a nested design, the concentrations (0, 10, 20, 30, 40, 50, and 60%) were nested within the calabur tree leaf extract methods (boiling and fractionation). The results showed that boiled calabur leaves extract was more effective than fractionated calabur leaves extract to inhibit growth of *C. gloeosporioides*. Boiled calabur leaves extract and fractionated leaves extract at various concentration showed capability to inhibit the growth, sporulation and viability of *C. gloeosporioides*. Boiled calabur leaves extract at different concentration levels were able to suppress disease development of papaya anthracnose disease on papaya fruit. The higher concentration of calabur leaves extract, are more effective to inhibit *C. gloeosporioides*.

Key words: anthracnose, *Colletotrichum gloeosporioides*, phytofungicide, calabur tree leaf extract

INTRODUCTION

The papaya anthracnose caused by *Colletotrichum gloeosporioides* is one of the limiting factors in papaya production. *C. gloeosporioides* has been reported to cause disease on most of the papaya plant such as leaf, stem, and fruit, both in pre and postharvest (Haggag & Singer, 2013). The pathogen was rapidly evolve and disperse in the rainy season, that was assisted by wind and rain splashes. *C. gloeosporioides* could infect the fruit surface through wounds, so that it can accelerate the onset of early symptoms. The symptoms on young fruit was initially recognized as small wound with thickening sap and dark small circles. When the infected fruit is ripe, the circles will develop and become a hollow (Maeda & Nelson, 2014).

Fungicide has been reported to be one of the effective method to control anthracnose on papaya (Maeda & Nelson, 2014). However, long term application of fungicide will cause negative effect on the health and environment (Damalas, 2009; Carvalho, 2017). Biopesticide derived from plant extract which is also known as phytopesticide, becomes one of the alternative methods to reduce the use of pesticides (Tripathi, 1998; Hikal *et al*., 2017; Kamble *et al*., 2016). The phytopesticide is prepared by extracting part of plants, processed, or made into concentrates that will not change their chemical structure. The use of phytopesticide in controlling anthracnose is expected to be more effective and safe. Phytopesticide is easy to develop (Dougoud *et al*., 2019) and it also has more advantage since the ingredients are cheap and easy to obtain (Tripathi, 1998; Hikal *et al*., 2017). One of the plant material that has the potential as a phytopesticide is *Muntingia calabura* which is also known as calabur tree (English) or kersen or talok (Indonesia) (Zakaria *et al*., 2007).

The leaves of calabur tree contain a group of flavonoid, tannin, alkaloid, and saponin (Zakaria *et al*., 2007) which has been reported as antifungal against *C. capsici*, *Rhizoctonia solani*, and *Fusarium oxysporum* (Khan & Nasreen, 2010). Thus, the leaves extract also may has capability to inhibit the growth of *C. gloeosporioides.* The research aimed to determine the ability of calabur tree leaf extract in inhibiting growth, sporulation, and viability of *C. gloeosporioides* as well as incubation period and disease severity on the fruit of papaya.

MATERIALS AND METHODS

Research Site. The research was conducted at the Laboratory of Plant Disease, Faculty of Agriculture, University of Lampung, from January to July 2019.

Experimental Design. The study on the potential of calabur tree leaf extract to inhibit growth, sporulation, and viability of *C. gloeosporioides* in vitro was arranged using nested completely randomized design with four replicates. The leaves extract concentrations (0, 10, 20, 30, 40, 50, and 60%) were nested within the extraction methods (boiling and fractionation). The treatments consisted of no boiled calabur tree leaves extract (0% concentration) (E1K0), boiled calabur tree leaves extract 10% (E1K1), 20% (E1K2), 30% (E1K3), 40% (E1K4), 50% (E1K5), 60% (E1K6), no fractionated calabur tree leaves extract (0% concentration) (E2K0), fractionated calabur tree leaves extract 10% (E2K1), 20% (E2K2), 30% (E2K3), 40% (E2K4), 50% (E2K5), and 60% (E2K6). The extraction method which had capability to inhibit growth of *C. gloeosporioides* in vitro study was further investigated on its capability to suppress the disease on papaya in vivo. Precisely, the study was performed to reveal the influence of calabur tree leaves extract on incubation period, disease severity, and AUDPC (area under the disease progress curve) of *C. gloeosporioides* on the papaya fruit. This experiment was arranged using randomized completely block design with five replication. Mean value among treatments were tested by orthogonal polynomial test ($\alpha = 5\%$).

Preparation of Calabur Tree Leaf Extract. The 200 g of freshly-old calabur tree leaves were washed in running water. The leaves were then mashed using blender machine with 1000 mL of distilled water and incubated for 24 h in room temperature. After incubation, 500 mL of calabur tree leaves extract suspension was boiled for 10 min. The remained 500 mL calabur tree leaves extract suspension was fractionated using a simple fractionation method performed by Efri *et al*. (2017). The charcoal was added in the fractionation device as a filter to separate polar and non-polar compounds. In this fractionation process, active polar compounds will be obtained. The results of boiled and fractionated were extracts of stock (*aliquots*). The boiled and fractioned calabur leaves extract were then diluted in each of the concentration needed in the treatments.

Potential of Calabur Tree Leabes Extract to Inhibit Growth, Sporulation, and Viability of *C. gloeosporioides* In vitro. *C. gloeosporioides* was cultivated on potato sucrose agar (PSA) media (200 g of potato, 20 g of sucrose, 20 g of agar, 1000 mL aquadest) that had been mixed with the leaves extract in each different concentrations. One mycelial plug (5 mm in diameter) of 7 d old of *C. gloeosporioides* was put on sterile petridish containing 10 mL of PSA medium and then incubated for 7 d at room temperature. Observation was conducted on colony growth, sporulation, and spore viability of *C. gloeosporioides*.

Growth of the colony was calculated by using formula**:**

$$D = \frac{D1 + D2 + D3 + D4}{4}$$

D = diameter of *C. gloeosporioides* (cm)
D1, D2, D3, D4 = diameter of the measurement results from four different directions.

Sporulation was calculated using formula:

$$C = \frac{t}{n \times 0.25} \times 10^6$$

C = spore density of *C. gloeosporioides*
t = number of spore at observed sample square
n = number of sample square
0.25 = a constant of using middle square

Spore viability was calculated using formula:

$$V = \frac{g}{g + u} \times 100\%$$

V = spore viability (%)
g = number of germinated spore
u = number of ungerminated spore

Effect of Calabur Tree Leaves Extract on Incubation Period, Disease Severity, and Area Under the Disease Progress Curve (AUDPC) of *C. gloeosporioides* Inoculation. A half-ripe papaya fruit (700-1000 g in weight) was washed in running water and dried using tissue paper. After dried, the fruit then sprayed with 70% alcohol to sterilize the fruit surface. The surface of the papaya fruit was 10 times randomly stabbed using sterile needles with 7 cm in depth. The leaves extract with various concentration was then sprayed on papaya fruit and air dried for 2 h. After that,

10 mL spore suspension of *C. gloeosporioides* (10^8 spores/mL) was sprayed on the surface of the papaya fruit. The inoculated papaya was placed on a sterile tray and incubated at room temperature for 7 d.

Incubation period. Incubation period is the period needed for the pathogen to cause symptom. Observation was conducted every day after inoculation and the day which is the symptom firstly appeared was recorded.

Disease severity. Observation was carried out every day on the symptom which was emerged on the fruit after inoculation. The symptomatic area and the overall surface area of the papaya fruit were recorded. The area of each symptomatic point on the fruit is drawn on a transparent plastic with a marker. The drawn area was calculated using millimeter block. This method was also performed to calculate entire surface area of the fruit. The disease severity (DS) was measured using the formula (Nur *et al*., 2017):

$$DS = \frac{\text{The area of anthracnose symptoms on fruit surface}}{\text{The total area of fruit surface}} \times 100\%$$

Area under the disease progress curve (AUDPC). AUDPC was calculated using formula (Simko & Piepho, 2012):

$$AUDPC = \sum_{i-1}^{n-1}\left(\frac{Y_i + Y_{i+1}}{2}\right)\left(t_{i+1} - t_i\right)$$

AUDPC = area under the disease progress curve
Y_i = an assessment of a disease (percentage, proportion, ordinal score, etc.) at the t observation
t_i = time (in days, hours, etc.) at the i observation
n = the total number of observations

RESULTS AND DISCUSSION

Potential of Calabur Tree Leaves Extract to Inhibit Growth, Sporulation, and Viability of *C. gloeosporioides* In vitro. The result showed that boiled calabur leaves extract had better capability to inhibit growth of *C. gloeosporioides* than fractionated calabur leaves extract at 4−12 days after inoculation (DAI) (Table 1). However, both of the extract did not influence sporulation and spore viability of *C. gloeosporioides* (Table 2; 3).

Effect Concentration of Boiled Calabur Tree Leaves Extract on the Growth, Sporulation, and Spore Viability of *C. gloeosporioides*. The concentration levels of boiled calabur tree leaves extract showed different capability to inhibit the growth of *C. gloeosporioides*. Based on the Polynomial orthogonal test showed that at 12 DAI, concentration levels of boiled calabur tree leaf extract resulted linearly effect on the colony growth (Y= -0.0133x + 7.3583), sporulation (Y= -0.09x + 8.2286), and viability (Y= -0.9107x + 88.43) of *C. gloeosporioides* (Figure 1–3). This result revelaed that the higher concentration level showed the higher capability to inhibit the growth of *C. gloeosporioides*.

Effect Concentration of Fractionated Calabur Tree Leaves Extract on the Growth, Sporulation, and Spore Viability of *C. gloeosporioides*. The concentration levels of the fractioned calabur tree leaves extract showed resulted different effect on the growth of *C. gloeosporioides*. It formed a linear pattern on colony growth (Y = -0.0193x + 8.6762), sporulation (Y = -0.0825x + 7.8179), and spore viability (Y = -0.6952x + 78.468) of *C. gloeosporioides* (Figure 4–6). In line with the boiled leaves extract, it also can be concluded that the higher concentration level, the higher capability to inhibit the growth of *C. gloeosporioides*.

Table 1. The effect of boiled and fractionated calabur tree leaves extract againts the colony diameter of *C. gloeosporioides* (cm)

Treatment	Obsevation to- (DAI)										
	2	3	4	5	6	7	8	9	10	11	12
E1	0.61	1.00	1.38	2.16	2.81	3.56	4.19	4.93	5.64	6.35	7.09
E2	0.66	1.39	2.09	2.90	3.65	4.51	5.25	6.04	6.73	7.54	8.10
Significancy	ns	ns	*	*	*	*	*	*	*	*	*

E1: boiled calabur tree leaves extract, E2: fractionated calabur tree leaves extract, DAI: day after inoculation, *: significant at level of 5%, ns: non significant at level 5%.

Table 2. The effect of boiled and fractionated calabur tree leaves extract againts the spore density of *C. gloeosporioides*

Treatment	Spore density (spore/mL)
E1 (boiled calabur tree leaves extract)	5.55 x 10^6
E2 (fractionated calabur tree leaves extract)	5.33 x 10^6
Significancy	ns

ns: non significant at level 5%.

Table 3. The effect of boiled and fractionated calabur tree leaves extract againts the spore germination of *C. gloeosporioides* (%)

Treatment	Obsevation time (HAI)		
	4	6	8
E1 (boiled calabur tree leave extract)	19.25	49.17	61.11
E2 (fractionated calabur tree leaves extract)	16.00	50.75	57.61
Significancy	ns	ns	ns

HAI: hour after inoculation, ns: non significant at level 5%.

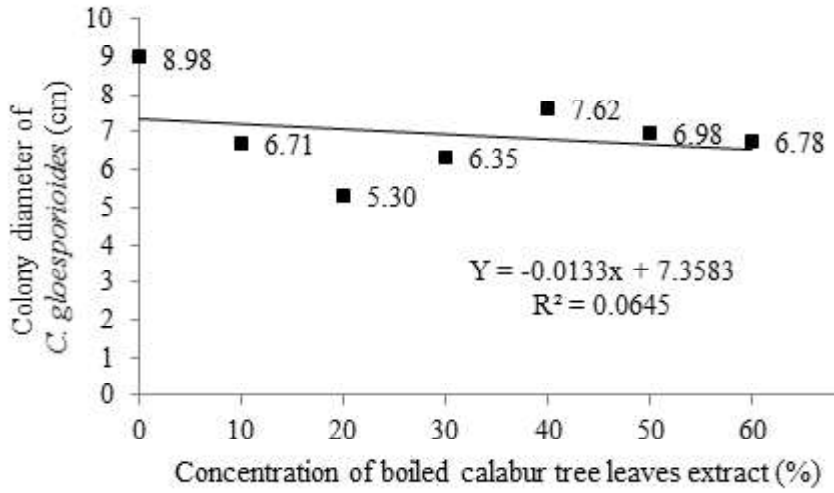

Figure 1. The colony diameter of *C. gloeosporioides* at the various concentration levels of boiled calabur tree leaf extract at 12 days after inoculation.

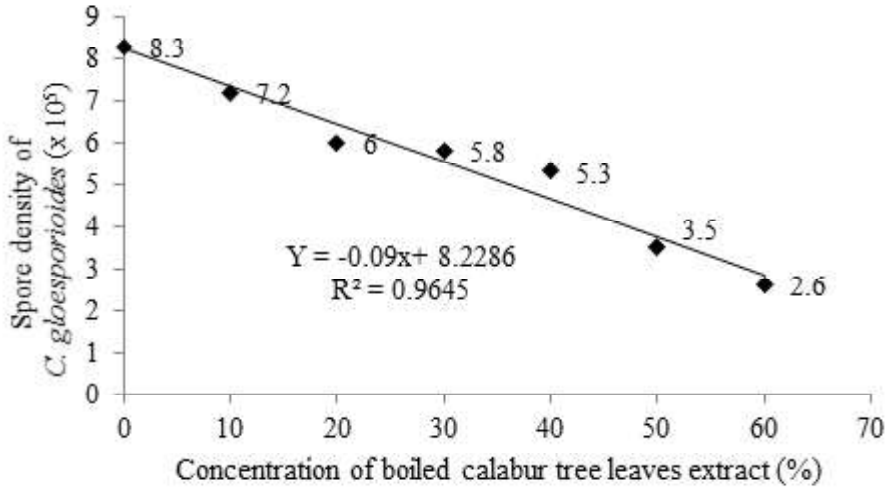

Figure 2. The spore density of *C. gloeosporioides* at the various concentration levels of boiled calabur tree leaves extract at 14 days after inoculation.

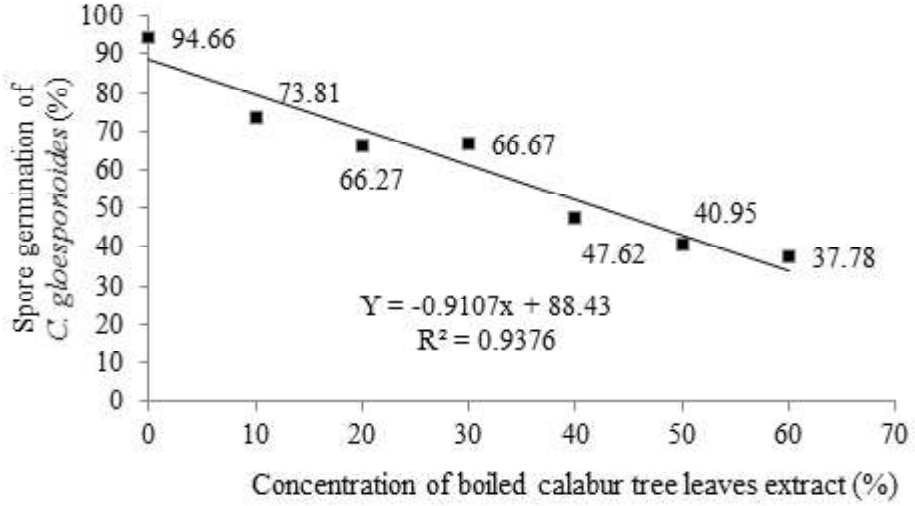

Figure 3. The spore germination of *C. gloeosporioides* at the various concentration levels of boiled calabur tree leaves extract at 14 days after inoculation.

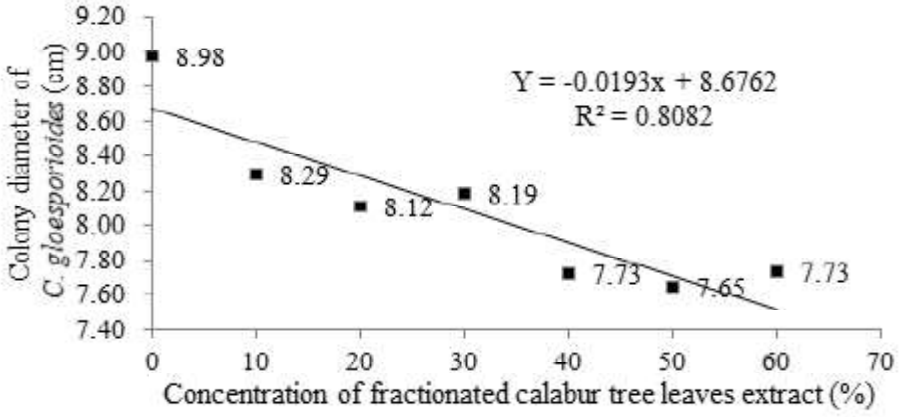

Figure 4. The diameter colony of *C. gloeosporioides* at the various concentration levels of fractionated calabur tree leaves extract at 12 days after inoculation.

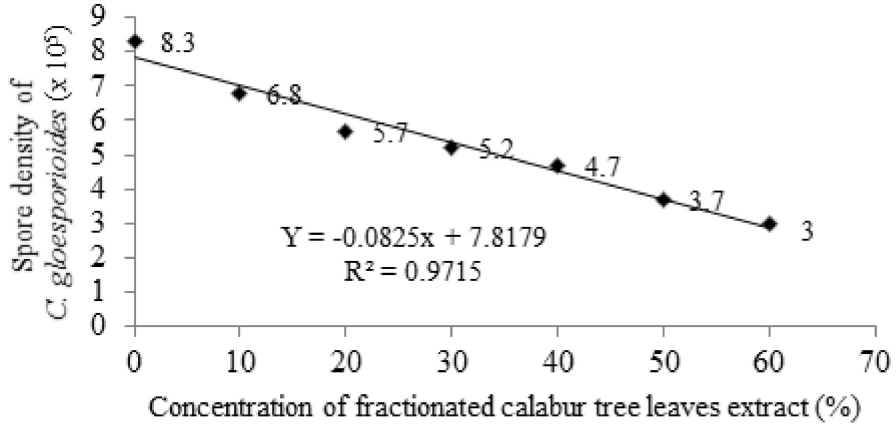

Figure 5. The spore density of *C. gloeosporioides* at the various concentration levels of fractionated calabur tree leaves extract at 14 days after inoculation.

The results of chosen method showed that the boiled calabur tree leaves extract have better capability to inhibit the growth, sporulation and spore viability of *C. gloeosporioides* than fractionated leaves extract. Thus, the leaves extract derived from boiling method was used for advanced investigation to inhibit disease development of papaya anthracnose on the papaya fruit.

The Effect of Boiled Calabur Tree Leaves Extract on the Incubation Period and Disease Severity on Papaya Fruit. Application of boiled calabur tree leaves extract with different levels of concentration did not significantly influence incubation period of the papaya anthracnose on papaya fruit (Table 4). However, application of boiled calabur tree leaves extract at

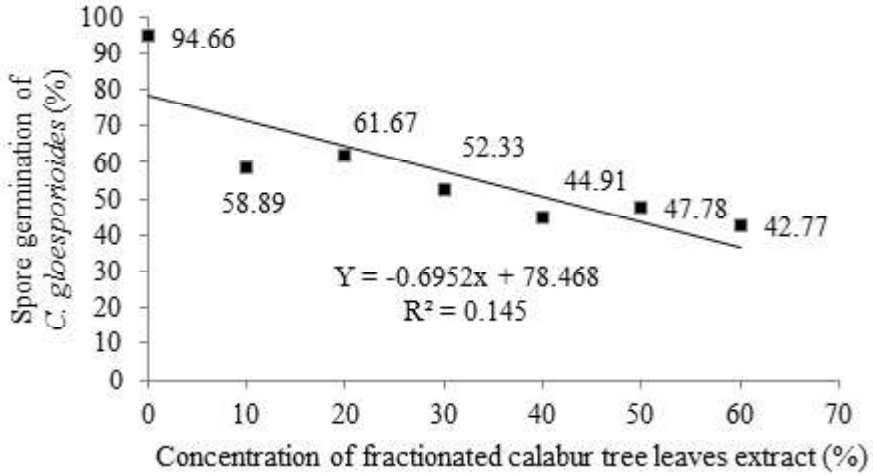

Figure 6. The spore germination of *C. gloeosporioides* at the various concentration levels of fractionated calabur tree leaves extract at 14 days after inoculation.

Tabel 4. The effect of boiled calabur tree leaf extract againts the incubation period of antrachnose disease on papaya fruit

Concentration (%)	Incubation period (d)
0	3.00
20	3.33
30	3.00
40	3.33
50	3.33
60	3.33
Significancy	ns

ns: non significant at level 5%.

Table 5. The AUDPC value of boiled calabur tree leaves extract effect againts the severity disease of anthracnose to time

Concentration (%)	Disease severity (%)	AUDPC value
0	31.54 a	96.12 a
20	28.14 a	86.93 a
30	27.09 ab	82.77 ab
40	22.31 b	68.43 b
50	8.36 c	30.58 c
60	7.98 c	25.45 c

The numbers in the same column followed by the same letters are not significantly different based on DMRT at the 5% level.

different concentration affected the disease severity (Table 5). It is shown on the linear pattern (Y = -0.4354x + 35.417) created by this application (Figure 7). It reveals that the higher concentration of calabur tree leaves extract, the lower of the disease severity. This phenomenon was thought to be related with the higher concentration of the extract that will be followed by the increase of the number of antifungal compounds presence in the extract.

The Effect of Boiled Calabur Tree Leaves Extract on the AUDPC. The results revealed that the boiled calabur leaves extract at a concentration of 60% resulted the lowest AUDPC value compared to other treatments (Table 5). Simko & Piepho (2012) stated that the lower AUDPC means that the given treatment was more effective in controlling pathogens. Here we determined that boiled calabur leaves extract with a concentration of 60% was the best concentration that can be used to suppress the disease development of papaya anthracnose disease on papaya fruit.

Calabur leaves contain various active compounds such as flavonoids, tannins, saponins, alkaloids (Zakaria *et al*., 2007; Siddiqua *et al*., 2010; Rajesh *et al*., 2014), and cathecin (Surjowardojo *et al*., 2014). These compounds are polar (flavonoids, tannins, saponins, and cathecins) and non-polars (alkaloids) (Harborne, 1984) compounds that has been reported to have capability as antifungal against several plant pathogens such as *C. capsici*, *R. solani*, and *F. oxysporum* (Khan & Nasreen, 2010; Singh & Kumar, 2013). The results in this study showed that boiled calabur leaves extract had better inhibition ability than fractionated leaves extract. This was thought to be related to the presence of different chemical compounds between leaves extract derived from both extraction methods. The active ingredients in leaves extracts was strongly influenced by temperature. Puspita & Desrita (2019) reported that there was a change of the bioactive compounds of *Excoecaria agallocha* mangrove leaf samples which were dried and to those which were boiled. The boiled calabur leaves extract is thought to contain alkaloids and tannins, meanwhile, the fractionated calabur leaves extract (using water as solvent) was thought to contain polar bioactive compounds such as flavonoids, tannins, saponins and cathecin.

Alkaloids has been reported as antifungal which can inhibit the biosynthesis of nucleic acids (Mazu *et al*., 2016). Flavonoids has been confirmed as antifungal, antimicrobial, antiviral, anticancer, and anti-insecticide (Górniak *et al*., 2019). Saponins was a compound which has capability as antifungal, causing lysis of microbial cells which can disturb the stability of the cell membrane (Tagousop *et al*., 2018). We settled that it supposed that the bioactive material of the non-polar alkaloid compound was more powerful than those the polar compounds (flavonoids, tannins, saponins and cathecins) on their capability to inhibit *C. gloeosporioide*. According to Singh & Kumar (2013), alkaloid compounds extracted from the leaves of the *Euphorbia hirta* has been reported having better ability to inhibit spore viability of *F. oxyporum* than flavonoids.

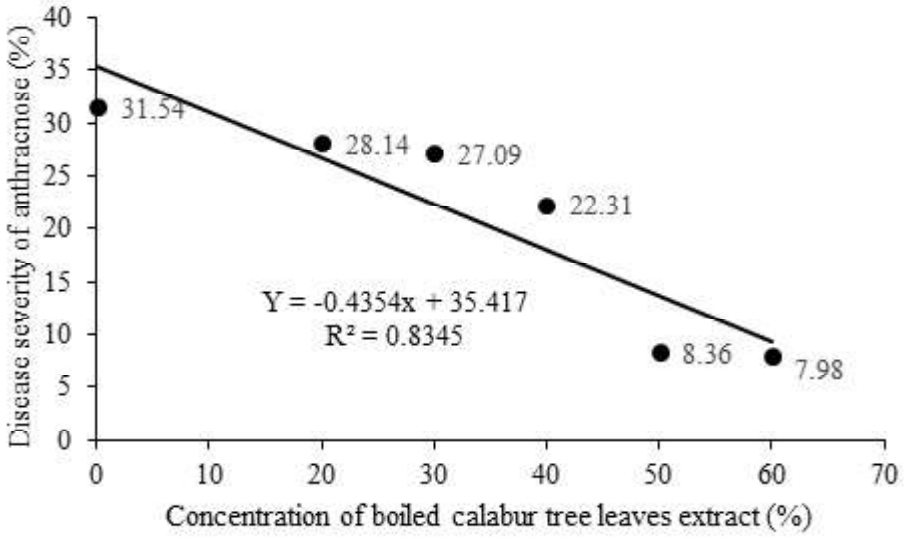

Figure 7. The disease severity of anthracnose at the various concentration levels of boiled calabur tree leaaves extract

CONCLUSION

Boiled calabur leaves extract was more effective than fractionated calabur leaves extract to inhibit growth of *C. gloeosporioides*. Boiled calabur leaves extract and fractionated leaves extract at various levels of concentration showed capability to inhibit the growth, sporulation and viability of *C. gloeosporioides*. Boiled calabur leaves extract at different concentration levels were able to suppress disease development of papaya anthracnose disease on papaya fruit. The more concentration of calabur leaves extract, the more effective to inhibit *C. gloeosporioides*.

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