International Journal of Advanced Research in Engineering and Technology (IJARET)
Volume 11, Issue 10, October 2020, pp. 209-217, Article ID: IJARET_11_10_021
Available online at http://www.iaeme.com/IJARET/issues.asp?JType=IJARET&VType=11&IType=10
ISSN Print: 0976-6480 and ISSN Online: 0976-6499
DOI: 10.34218/IJARET.11.10.2020.021

FIRST REPORT OF EFFICACY STUDY OF BIO-EXTRACT TO CONTROL PESTALOTIOPSIS SP. AFFECTING PARA RUBBER LEAF DISEASE (HEVEA BRASILIENSIS MUELL. ARG) UNDER CLIMATE VARIABILITY

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ABSTRACT

Academic and industrial research experiments designed to stop the outbreak of Para leaf diseases caused by Pestalotiopsis sp. have been conducted in recent years in several nations. This manuscript reports a December 2019 to March 2020, study held in Thailand. This research tested the bio-efficacy of a natural bio-extract (YRU 1) fertilizer to fight Pestalotiopsis sp. in Natural Rubber (NR) plant leafs. Researchers conducted two separate experiments, in vitro and in vivo, using a mixture of distilled water and percentages of four natural ingredients. Results indicate that the bio-extract at 0.50% concentration level significantly decreased the number of Pestalotiopsis sp. colonies, in both in vitro and on rubber plant leaf (in vivo). Additionally, the use of the same concentration reduced fungal growth during new leaf development. The results suggest that the bio-extract used in these experiments is effective in reducing and controlling the direct development and growth of Pestalotiopsis sp. on leaves. It was suggested that a fluctuate in the climatic factors caused a negative impact on the leaf disease of rubber in southern Thailand. Use of natural bio-extracts therefore, may be *used as an alternative to the toxic chemicals that threaten humans, animals and the surrounding environment.*

Key words: Natural rubber, bio-extract, *Pestalotiopsis* sp., Para leaf disease

Cite this Article: Isma-ae Chelong, Johnny J Moye, Ajaman Adair and Surachai Bonwanno, First Report of Efficacy Study of Bio-Extract to Control Pestalotiopsis Sp. Affecting Para Rubber Leaf Disease (Hevea Brasiliensis Muell. Arg.) Under Climate Variability, *International Journal of Advanced Research in Engineering and Technology*, 11(10), 2020, pp. 209-217.
http://www.iaeme.com/IJARET/issues.asp?JType=IJARET&VType=11&IType=10

1. INTRODUCTION

Natural rubber (NR) is a natural polymer obtained from the trees of the *Hevea brasiliensis* species. Possessing excellent mechanical and physical properties, NR is a natural (green) resource having a wide range of applications (Hamzah et al., 2012). As the source of more than 40,000 products, NR is used by consumers' world wide. Natural rubber is one of the most important polymers produced by plants. Thailand is the world's number one exporter of NR (Thailand Convention and Exhibition Bureau (TCEB), 2019). Harvesting, producing, and exporting rubber has a large impact on Thailand's economy.

The NR industry is facing a critical challenge. The *Pestalotiopsis* sp. fungal disease is devastating rubber trees. Bhanumathi and Ravishankar Rai (2007) reported that the *Pestalotiopisis* sp. is a major pathogen causing the leaf blight disease, *Syzygium cumini*. The disease makes rubber tree leaves fall from its branches. This problem is difficult to contain because the wind can easily spread this leaf falling disease. Since the rubber authority of Thailand (RAOT) detected the fungus in September 2019, the disease has ravished more than 700,000 rais (1.12 trillion square meters) of rubber fields in and around the Naratiwat, Pattani, Yala, Songlka provinces of southern Thailand. Rubber output in these four provinces has dropped by 40,000 tonnes, almost 50% of the normal production. The devastation caused by *Pestalotiopisis* sp. is not confined to Thailand. Between September 2019, and June 2020, the *Pestalotiopsis* sp. fungus has damaged 2.3 million rai (3.73 trillion square meters) in Indonesia, and 16,000 rai (25.6 billion square meters) in Malaysia. The International Rubber Research and Development Board (2019) reports that India and Sri Lanka are also experiencing this destructive fungus in rubber fields located in those countries (Bangkok Post, 2019).

To combat the *Pestalotiopisis* sp. fungus problem, Bhanumathi and Ravishankar Rai (2007) recommends the use of Bavistin and Roko fungicide chemicals at a minim concentration of 50mg/L to inhibit growth of the *Pestalotiopisis* sp. fungus. The International Rubber Consortium Limited (IRCo), (2019) reports that Thailand is using drone technology to spray chemicals on NR plantations to stop the spread of the *Pestaotiopisis* sp. fungus (Rubber and Plastics News, 2019). The use of drone technology to apply chemicals to NR plants is problematic. The inability to directly apply the chemicals to NR plant leafs by drone, requires the use of a great deal of chemicals to fight this Para leaf disease. Treatments therefore are expensive. With the incorrect amount of chemical application, fungicide-resistant strains of the disease could develop. Fungicides are toxic and have undesirable effects on humans, animals, and ecosystem (Ashwani et al., 2011). When spraying chemicals from drones, the health and safety of environmental organisms must also be considered. Several studies claim an improvement in bio-efficacy when using bio-extracts as a fungicide. Elmer et al. (1994) demonstrated the importance of anti-fungal phenolic compounds in inhibiting various fungal pathogens. Sunpapao and Pornsuriya (2013) reported that bio-extract chitosan directly inhibits the growth of *P. botryosa*, one causal agent of Para rubber leaf fall disease that withers its mycelia and zoospores. Tanapichatsakul et al. (2019) reported that eugenol, myristaldehyde, lauric acid, and caprylic acid were the primary antimicrobial and antioxidant compounds in both crude extracts. This was the first report of eugenol being a biologically active compound of *Neopestalotiopsis* sp. and *Diaporthe* sp. fungal endophytes. The Ayoub and Niazi (2001) and Elsharkawy and El-Sawy (2015) studies found that plant extracts were effective bio-control agents that combat a wide range of plant pathogens. Plants have the ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins. Cowan (1999) reviewed and reported the components with phenolic structures, like carvacrol, eugenol and thymol were highly active against pathogens. Shabana et al. (2017) reported that the efficacy of eight plant extracts (garlic, clove, garden quinine, Brazilian pepper, anthi mandhaari, black cumin, white cedar and neem) controlled leaf rust disease in wheat, when investigated in vitro and in vivo. That study specifically found in vitro treatments inhibited spore germination by more than 93%. Neem extract recorded 98.99% inhibition of spore germination. The results of using neem is not significantly different from what can be expected from the fungicide *Sumi-8*, which inhibits 100% spore germination. Farnaz Abed-Ashtiani et al. (2012) found that levels of disease in rice were associated with silicification and fortification of leaf epidermal cells through silicon fertilization. These studies suggest that natural plant extracts can produce the same results as fungicides without the negative effects on humans, animals and environment.

2. STUDY OBJECTIVE

The objective of this study was to investigate the effects of a natural bio-extract (YRU 1) to inhibit the growth of *Pestalotiopsis* sp. on natural rubber Para leaf disease.

3. MATERIALS AND METHODS

The bio-extract used in this experiment was prepared in the Laboratory of Biology, faculty of Science, Technology, and Agriculture at the Rajabahat Yala University, Yala, Thailand.

3.1. Bio-extract preparation

The researchers created a 100% natural substrate, which will be referred to as *bio-extract fertilizer*. This fertilizer is composed of wood vinegar, propolis, tar and zinc oxide. The four ingredients were placed and mixed in a sterile closed plastic container and stored at room temperature.

3.2. Testing for antifungal efficiency in vivo

To find the effective bio-extract concentration to inhibit *Pestalotiopsis* sp. growth, seven PDA plates were prepared. Following the Laflamme et al. (2000) process, PDA plates were prepared with a solution of sterilized distilled water with the following percentages of bio-extract: (T1: (control), T2: 0.125%, T3: 0.25%, T4: 0.50%, T5: 0.75%, T6: 1.00%, T7: 1.25%). Three replicate plates of each treatment for each bio-extract concentration were inoculated in the center with a plug (5 mm diameter) from the edge of a 3-5 day-old *Pestalotiopsis* sp. colony. A PDA plate containing only sterile distilled water served as the control. Colony sizes were measured seven, 24, 48 and 72 hours after the inoculations. Percent inhibition of diameter growth (PIDG) was calculated from treatment-average diameters in experiments using the following formula:

Percent inhibition of diameter growth (PIDG) = {[A - B]/A]}/ 100

Where A is the diameter of control colonies, and B is the diameter of treated colonies.

3.3. Testing for antifungal efficiency in vitro

The researchers used the following process to test for an effective concentration of bio-extract to inhibit the growth of *Pestalotiopsis* sp., in vitro. Rubber tree trunks and leaves were sprayed with bio-extract at treatment of 1 to 7 (T1: (control), T2: 0.125%, T3: 0.25%, T4: 0.50%, T5: 0.75%, T6: 1.00%, T7: 1.25%). Fifty ml of bio-extract solution was also sprayed within 1.5 meters around each tree used in the experiment. The inhibition rates were measured at 2, 4, 6, 8, 10, 12 and 14 weeks. A zoospore suspension was obtained by rinsing with 15 ml sterilized distilled water into culture plates with *Pestalotiopsis* sp. Culture plates were incubated at 4°C for 15 minutes and then placed at room temperature for 30 minutes to release zoospores. Zoospores were counted and analyzed. The lesions from *Pestalotiopsis* sp. penetration in leaves were measured. Percent inhibition was calculated using the percent inhibition of diameter growth (PIDG) formula used in the in vivo experiment.

3.4. Climatic factors

Climatic factors data on temperature, precipitation and wind were recorded using a data online.

3.5. Statistical analyses

The completely randomized design (CRD) method was repeated twice for each experiment. Data were analyzed by analysis of variance (ANOVA), and statistical significance of mean differences between chitosan and control treatments was assessed with Duncan's Multiple Range Test (DMRT) for multiple comparisons.

4. RESULTS

4.1. Antifungal efficiency of bio-extract in vivo

Effect of bio-extract concentration on inhibition of Pestalotiopsis sp. on media

In order to determine the inhibitory effect of bio-extract on radial growth of *Pestalotiopsis* sp., agar plates with seven treatments were prepared and tested against this fungus pathogen: (T1: (control), T2: 0.125%, T3: 0.25%, T4: 0.50%, T5: 0.75%, T6: 1.00%, T7: 1.25%). The PDA amending with sterile distilled water served as control, and this in fact showed growth differences to a PDA medium. All experiment treatments, 2 to 7, reduced the growth of *Pestalotiopsis* sp. over 7-day post inoculation. Growth inhibition consistently increased with treatment 4 (T4: 0.50%). Table 2 identifies the effect of bio-extract on *Pestalotiopsis* sp. The effects were statistically significant, as detailed in Table 2. However, the growth inhibition percentage rate at the first stage increased as the bio-extract concentrations were increased. The highest values were reached at treatments T4 and slightly decreased at T5 before taking plateau as it appears in Table 2. Figure 1 shows inhibition curves of *Pestalotiopsis* sp. function by treatments at various rates and time intervals.

Table 2 Effect of bio-extract concentration on percent inhibition of *Pestalotiopsis* sp. on media

Treatment *(bio-extract concentration)*	**Inhibition rate (%)**		
	24 hrs.	**48 hrs.**	**72 hrs.**
T1: (control)	2.32±1.18c	2.02±1.21f	1.85±1.76g
T2: 0.125%	24.84±1.38b	18.14±2.10e	12.26±2.36f
T3: 0.25%	68.37±1.47a	54.87±1.56d	38.21±2.78e
T4: 0.50%	95.81±2.26a	90.11±1.89a	68.45±2.65a
T5: 0.75%	95.37±1.54a	70.54±2.34b	52.78±2.10b
T6: 1.00%	95.38±1.67a	69.47±2.40b	49.78±2.12c
T7: 1.25%	95.39±1.21a	68.47±1.87c	41.85±2.32d

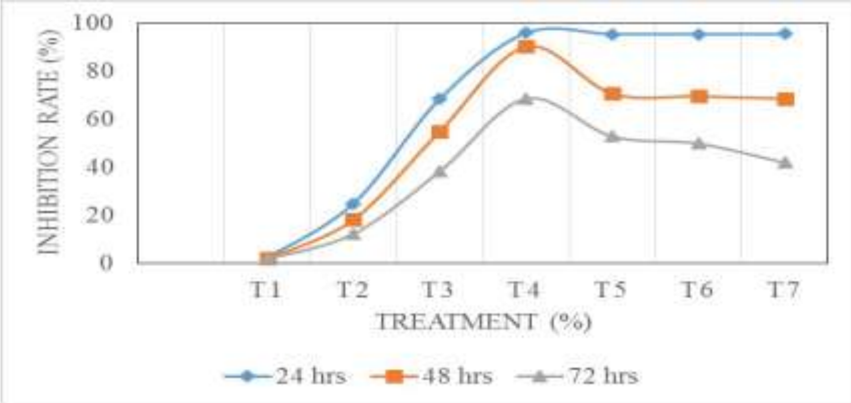

Figure 1. Inhibition curves of *Pestalotiopsis* sp. function by treatments over time

4.2. Antifungal efficiency of bio-extract in vitro

Effect of bio-extract concentration on percent inhibition of Pestalotiopsis sp. on Para rubber leaves

A bio-extract treatment of T1 to T7 were sprayed on the Para rubber plantation trees trunk, and within 1.5 meters around the trees. The inhibition rates were measured at 2, 4, 6, 8, 10, 12 and 14 weeks. A concentration percentage treatment of 0.50%(T4) showed 95% inhabitation rate to *Pestalotiopsis* sp. The effect of bio-extract on inhibition of *Pestalotiopsis* sp. invasion to Para rubber leaves can be seen on Table 2. The details of the statistical significant effect of the growth inhibition is shown in Table 3. The growth curves for actual treatments are below that for control as shown in Figure 2.

Table 3 Effect of bio-extract concentration on percent inhibition of *Pestalotiopsis* sp. on Para rubber leaves

Treatment *(bio-extract concentration)*	**Inhibition rate (%)**						
	2weeks	**4weeks**	**6weeks**	**8 weeks**	**10 weeks**	**12 weeks**	**14weeks**
T1: (control)	-	-	-	-	-	-	-
T2: 0.125%	0.623f	0.57f	0.41f	0.36f	0.24f	0.20f	0.14f
T3: 0.25%	12.15e	11.45e	10.30e	10.02e	9.54e	8.04e	7.32d
T4: 0.50%	98.44a	90.87a	87.12a	82.52a	80.78a	79.24a	78.39a
T5: 0.75%	95.33b	85.45b	77.47b	64.52b	51.21b	42.78b	30.33b
T6: 1.00%	90.65c	74.21c	65.12c	51.12c	30.45c	18.02c	14.36c
T7: 1.25%	87.54d	62.74d	54.12d	30.78d	25.41d	10.45d	9.78

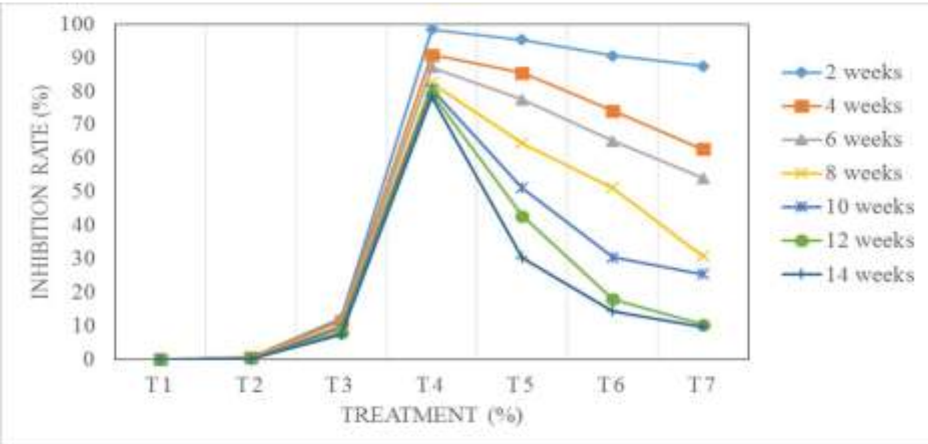

Figure 2 Percent of inhibition with various treatments of bio-extract over time.

The objective of this study was to investigate the effects of a natural bio-extract fertilizer to inhibit the growth of *Pestalotiopsis* sp. on natural rubber Para leaf. Previous studies indicate that natural plant extracts were successful in combating plant disease. Bio-extracts may fight plant pathogens either by inhibiting fungal growth or aiding plant resistance to those pathogens. Discovering alternate methods may decrease the use of chemicals, presenting less risk to humans, animals and the environment.

Researchers from the Laboratory of Biology, faculty of Science, Technology, and Agriculture at the Rajabhat Yala University, Yala, Thailand conducted this study. Researchers conducted in vivo and in vitro experiments using a natural bio-extract composed of 80-85% wood vinegar, 5-10% propolis, 1-5% tar, and 0.5-1% zinc oxide. Bio-extract solutions were created as T1 (control), T2: 0.125%, T3: 0.25%, T4: 0.50%, T5: 0.75%, T6: 1.00%, T7: 1.25%). Each in vitro test, using different bio-extract percentages showed reduced growth of *Pestalotiopsis* sp. over 7-day post inoculation period. The treatment using 0.50% bio-extract fertilizer solution produced significant inhibition of *Pestalotiopsis* sp.

In the in vivo test, rubber tree trunks and leaves were sprayed with bio-extract at a treatment of fifty ml of bio-extract solution. The solution was also sprayed within 1.5 meters around each tree used in the experiment. The inhibition rates were measured at 2, 4, 6, 8, 10, 12 and 14 weeks. A zoospore suspension was obtained by rinsing with 15 ml sterilized distilled water into culture plates with *Pestalotiopsis* sp. Culture plates were incubated at 4°C for 15 minutes and then placed at room temperature for 30 min to release zoospores. Zoospores were counted and analyzed. The lesions from *Pestalotiopsis* sp. penetration in leaves were measured. Percent inhibition was calculated using the percent inhibition of diameter growth (PIDG) formula used in the in vivo experiment. As was in the in vitro test, the treatment using 0.50% bio-extract fertilizer solution produced significant inhibition results.

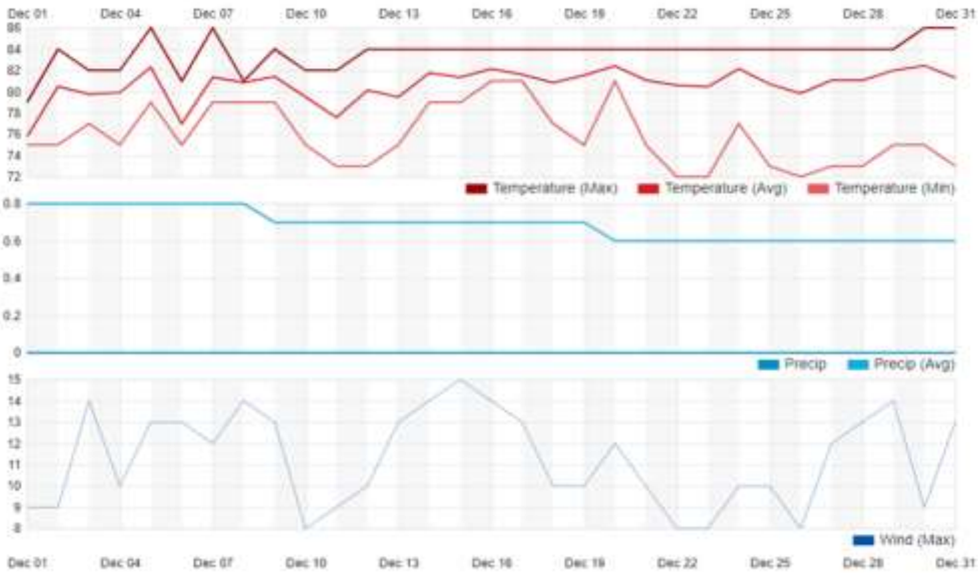

Figure 3 Monthly temperature, precipitation and wind in December 2019.

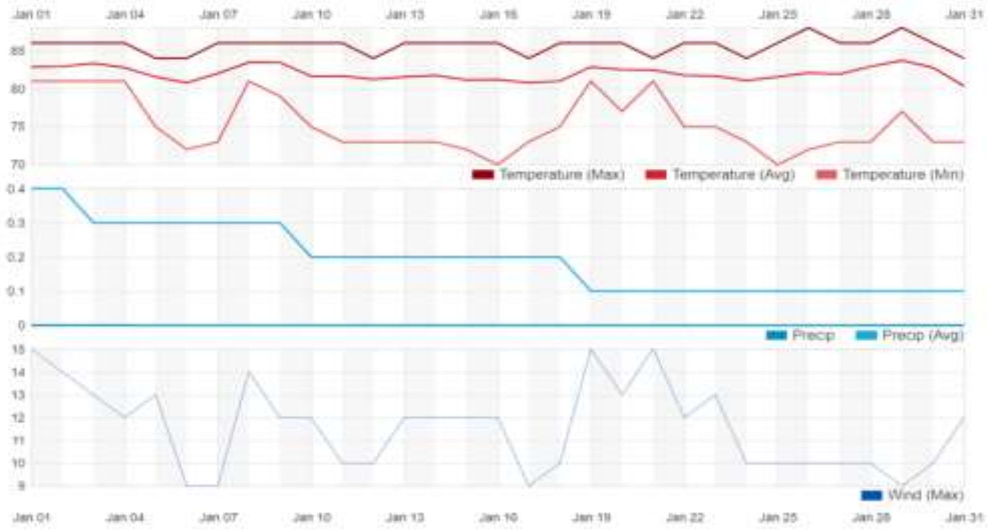

Figure 4 Monthly temperature, precipitation and wind in January 2020.

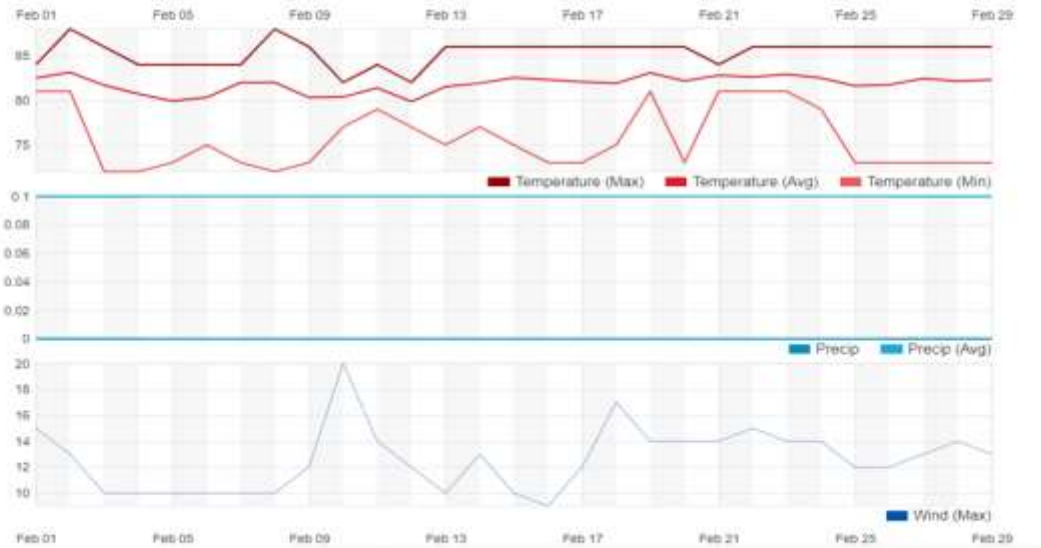

Figure 5 Monthly temperature, precipitation and wind in February 2020.

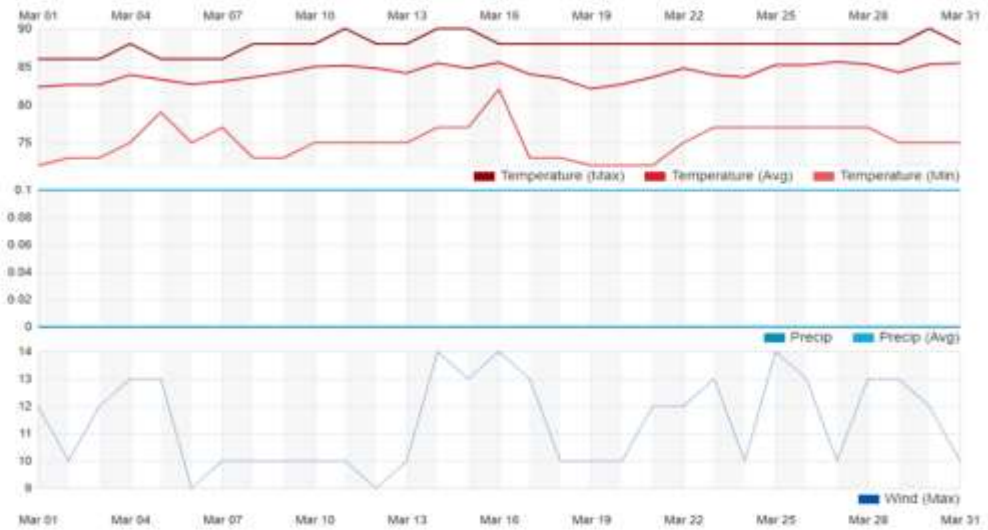

Figure 6. Monthly temperature, precipitation and wind in March 2020.

5. DISCUSSION

The southern region of Thailand has recently experienced Para rubber leaf fall caused by *Pestalotiopsis* sp. Growth of this fungus has been exacerbated by heavy and variability of rain falls, temperature and wind (Figure 3-6) cause of spreader of spore experienced in 2019 and 2020. Heavy rains allow the fungus to propagate quickly, destroying many natural rubber trees. Destruction of rubber trees presents a critical problem for Thailand's economy (Bangkok Post, 2019).

Studies have found natural alternatives in combating various plant diseases. This study found that a solution of natural ingredients can be used to fight the *Pestalotiopsis* sp. Para leaf disease on natural rubber trees. Additional research for the control of *Pestalotiopsis* sp. using bio-extract is needed. The objective of this study was to investigate the effects of a natural bio-extract to inhibit the growth of *Pestalotiopsis* sp. on natural rubber Para leaf. Two separate experiments using a 100% natural substrate composed of wood vinegar, propolis, tar, and zinc oxide. These ingredients were mixed in distilled water and applied in vitro and in vivo.

During the in vitro test, the researchers found the growth rate of *Pestalotiopsis* sp. was reduced in PDA plates amended with bio-extract fertilizer at a concentration of 0.50%. The in vivo test showed that the bio-extract sprayed on trunks, leaves, and the ground around the rubber tree significantly inhibited growth of *Pestalotiopsis* sp. on Para rubber leaves (Table 3). The results of both in vitro and in vivo experiments suggest that bio-extract fertilizer is an effective means to combat *Pestalotiopsis* sp. The results of these experiments suggest that the bio-extract used in this study is successful in controlling plant pathogenic fungi, therefore can be used as an alternative to using chemical treatments (Bajwa et al., 2004 : Rahber-Bhatti, 1998). Using this bio-extract would be an organic and ecofriendly tool to manage *Pestalotiopsis* sp. leaf disease of rubber tree Para leafs (Tapwal et al., 2011). Researchers should conduct similar research to test and replicate these results.

6. CONCLUSIONS

The efficacy of bio-extract against *Pestalotiopsis* sp. fungus was determined in this study, thanks to the complementarity of analyses conducted in vitro and in vivo (planta). It could be concluded that the bio-extract solution used in this study is useful to control Para rubber leaf disease. This experiment and other research reports identify bio-extracts are a means to control plant pathogenic fungi. Bio-extracts hold promise for the organic and ecofriendly management of leaf diseases of Para rubber. Additional studies and findings of those studies are necessary and would become the foundation for the use of bio-extract agents as a safe and cost-effective control against of *Pestalotiopsis* sp. causing rubber leaf disease of Para rubber tree.

ACKNOWLEDGMENTS

Authors are grateful to the YRU Research Fund for financial support and also appreciate the kind support from the Faculty of Science technology and agriculture, Yala Rajabhat University, Yala, Thailand.

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