Evaluation of Selected Chemical Pesticides for Controlling Bacterial Heart Rot Disease in Pineapples Variety MD2

S Sidik¹, Z Sapak²

¹ Centre of Postgraduate Studies, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Jasin Campus, 77300 Merlimau, Melaka, Malaysia.
² Crop Protection Research Group, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA. 45400 Shah Alam, Selangor, Malaysia.

Corresponding author’s e-mail address: zaiton3338@uitm.edu.my

Abstract. Bacterial heart rot (BHR) disease in pineapple variety MD2 is caused by Dickeya zeae syn. Erwinia chrysanthemi. The present study aims to screen the effectiveness of four systemic chemical pesticides in different dosages for controlling the BHR pathogen in vitro and to evaluate the effectiveness of delivery techniques of the selected chemical pesticides based on in vitro results to control the disease under rain shelter conditions. The pathogen was isolated from symptomatic pineapple leaves with the appearance of water-soaked and rotten basal tissues and confirmed via pathogenicity test. A poisoned food technique was used for in vitro assessment. The pathogen was tested with different pesticide concentrations of 250, 500, 750, 1000 and 1500 mg L⁻¹. Two fungicides were selected based on in vitro study for further investigation under the rain shelter condition. This in vivo study was performed with arrangement of complete randomize design with five treatments and 10 replicates of pineapple plants per treatment. The treatments were difenoconazole with pathogen, mancozeb with pathogen, benomyl with pathogen (standard industry), positive control (pathogen only) and negative control (without pathogen). Data of disease incidence (DI) and disease severity index (DSI) were collected for six-weeks after the early symptoms of BHR were detected. Finding of in vitro study showed that mancozeb and difenoconazole were able to control the pathogen at the lowest concentration of 250 mg L⁻¹ from the recommended rate. These two fungicides were consistently giving the similar results for the rain shelter study. The treatments with difenoconazole recorded the lowest value of DI and DSI with 4.60% and 0.00%, respectively. Then followed by mancozeb with DI of 5.62% and 63.71%. In contrast, besides positive control, the treatment of benomyl displayed the high values of DI (8.38%) and DSI (89.81%). Based on these findings, difenoconazole with combination method is the most effective control method for controlling BHR in pineapple. However, this research is highly recommended to be further investigated under the field conditions.

Keywords: Bacterial Heart Rot; Pineapple; Pesticides; Delivery Method

1. Introduction

Pineapple is a tropical fruit crop and known for health, juiciness, a robust tropical taste and tremendous wellness effects. The economic expansion of pineapple is mainly based on the stabilization of productivity by the world’s largest supplier of pineapple in Costa Rica. Malaysia was among the pineapple industry’s major players previously in the 1960s - 1970s, after Costa Rica and the Philippines. In the past, Malaysia was one of the three major pineapple producer countries, but
Malaysia’s status as the world’s leading exporter of pineapples has been taken over by other countries (DOA, 2017).

In 2013, the top 10 pineapple growers in the world were Costa Rica, Peru, the Philippines, Thailand, Indonesia, India, Nigeria, China, Mexico and Colombia which contributed 70.5% of the world’s pineapple production. Currently, Malaysia government is giving attention to boost the pineapple production by providing several tasks to the Malaysian Pineapple Industry Board to carry out. Pineapple production increased from 244,352.70 tons in 2013 to 340,721 tons in 2017. In 2018, 14,046.33 hectares of pineapple farms produced 322,459.52 metric ton of fruits along with other tropical fruits (DOA, 2018).

Like other crops, the production of pineapple has several challenges, and issues that need to be carefully tackled. Among the most popular insect pests targeting the pineapple crops are ants, mealybug, scales and souring beetles. The low production of pineapple fruits and plants can also be contributed by pathogenic microorganisms such as fungi, viruses and bacteria. The most common diseases that have been reported in pineapple are butt and black rot caused by a pathogenic fungus known as Ceratocystis paradoxa, fruitlet core rot by Penicillium spp., Fusariosis by Fusarium spp, virus wilt disease associated with mealybug and heart rot disease, either caused by bacterium Erwinia chrysanthemi or fungus Phytophthora spp.

Nowadays, the disease can be found in all planted areas of pineapple around the globe. In 2015, a group of researchers from Universiti Sains Malaysia reported that bacterial heart rot disease pathogen which was isolated from the infected pineapple plants is much closer to Dickeya zeae, based on phylogenetic analysis using multilocus sequence analysis. Bacterial heart rot disease can be recognized in the field as the infected pineapple plants would display the symptoms of the water-soaked lesion at the central of the leaf, then followed by the formation of browning around the leaf margin and mesophyll tissue. After a few days of initial infections at the meristem, apical tissue and bud shoot, the stem at the pineapple heart can be easily pulled out from the bottom of the plants.

In some conditions, infection on young pineapple fruit can be seen vividly when the fruit starts to soft rot and will rapidly collapse during the maturity stage. This infection is also known as pineapple fruit collapse disease. Usually, 3 to 8 months old pineapple plant is susceptible to the disease. Fosetyl aluminium has been widely used in Australia as a pre-dipping treatment for pineapple planting materials. Meanwhile, in Kerala, India, the most commonly used pesticides to control BHR on pineapple are Mancozeb, Carbendazim and Hexaconazole. To achieve the aim of the study, two objectives were explored which are to screen the effectiveness of systemic chemical pesticides with different dosages for controlling BHR pathogen in vitro and to evaluate the effectiveness of delivery techniques of the selected chemical pesticides to control BHR disease under rain shelter conditions (in vivo)

2. Material and Methods

2.1. In vitro study of chemical fungicides for control bacteria heart rot (BHR) disease

2.1.1. Isolation pathogen

The bacterial pathogen was isolated from the infected pineapple leaves in the laboratory under sterile condition. The leaf samples were then cut into small segment with 2 cm in length, soaked with 0.5% sodium hypochlorite for 3 minutes, rinsed with sterile distilled water twice, and dried on the sterile paper tissue in the laminar air flow for 15 minutes. Then, the dried sample and 10 ml of sterile distilled water were then ground in a sterile mortar. After 20 minutes, the suspension was put in the universal bottles to make a serial dilution.

2.1.2. Pathogenicity test

The bacterial isolate with characteristics of suspected pathogen was confirmed through physiological and biochemical testing. This bacterial isolate was then further confirmed as the pathogen with pathogenicity test. The bacterial suspension of suspected pathogen with 10^8 cfu was applied to the pineapple plants by pouring it to the 4 month-old pineapple suckers. The lesion of the midpoint of the
leaf was made by using sterile scalpels and 500 μL bacterial suspension was inoculated on the above of the slits of lesions. The transparent tape was used to cover the absorption ball that was immersed with the bacterial suspension for 24 hours.

2.1.3. Metabolic profiles (biology) analysis
The bacterial isolate was identified using a Biolog system. In this test, 24 hour old pure culture of the bacterial isolate was used. Carbon source utilization patterns were obtained for selected isolates using the Biolog MicroLog Microbial Identification System following the manufacturer’s instructions.

2.1.4. Fungicides
There are four types of fungicides with different active ingredients were selected and used in this experiment. They are difenoconazole 25%, EC (SIKOR), mancozeb 80%, WP (Kencobe M45), fosetyl –AL 80%, WP (Aliattes) and benomyl 50% WP (Benocide 50 WP).

2.1.5. Effect of fungicides on BHR pathogen in vitro
With some modification, using modified nutrient agar with different fungicide concentrations. A fresh bacterial pathogen culture was grown on tryptone soy agar for 24 hours and then the full loop of the culture was transferred into 10 ml of Nutrient water. The fungicide was produced as a poison agar in conjunction with nutrient agar. The nutrient agar was autoclaved at 110 °C for 30 minutes, then the different concentrations of the fungicides were completely dissolved by mixing with the nutrient agar for 5 minutes.

A control set of poisonous agar not supplemented with any fungicide was then prepared for comparison. The bacterial pathogen suspension was spread on each of the poisonous agars for each of the fungicides using a glass hockey stick. Each fungicide concentration was repeated four times, and this experiment was repeated twice as recommended by Wood, Fisher & Wang (2013).

2.1.6. Data analysis
Bacterial pathogen colonies grown on the poison agar were counted and their mean for each treatment was compared using one way ANOVA in SPSS V25 with the significant differences among the means (P = 0.05).

2.2. In vivo study of chemical fungicides for controlling bacteria heart rot disease in pineapple

2.2.1. Preparation sucker MD2 pineapple variety
A total of 100 pineapples 4-month-old MD2 suckers were purchased from FIMA Sdn Bhd, Kluang Johor, Malaysia. All plant units are maintained in accordance with the Malaysian Pineapple Industry recommended industry standard practices for pineapples. All pineapple plants are monitored daily to ensure that they are free from diseases and pests before being tested.

2.2.2. Experimental design
This study used a complete randomized design (CRD) to arrange all the experimental units per treatment in the rain shelter. The treatments were designed based on the result obtained from in vitro study (Table 2). Difenonconazole and mancozeb with 250 mg a.i/L of concentration were tested. Meanwhile, benomyl fungicide was included as a control treatment as pineapple industry still applies this fungicide to manage BHR in pineapple. Benomyl concentration used followed the standard industry for pineapple with concentration of 5.6 mg a.i/L. Meanwhile, for the application method of the fungicides, two methods which are dipping alone, and combination of dipping and spraying were applied. Besides, the effectiveness of fungicides tested, the delivery methods of either dipping alone or combination of dipping and spraying were also evaluated.

2.2.3. Dipping technique
Each experimental unit was dipped with the selected fungicide at a given concentration before planting. For each treatment of fungicide, the fungicide was prepared by diluting in 5L of tap water and then the basal of suckers was dipped for 10 mins in the fungicide and dried for 24 hours before
planting. After one month of planting the suckers, they were then inoculated with the BHR pathogen (Table 1).

Table 1. Treatment with pathogen and chosen fungicide.

| Treatment | Description |
|-----------|-------------|
| T1        | Pathogen with difenocanazole(250 mg a.i/L) |
| T2        | Pathogen with mancozeb(250mg a.i/L) (Control industry) |
| T3        | Pathogen with benomyl(5.6 mg a.i/L) (Control industry) |
| T4        | Pathogen alone without fungicide (Positive control) |
| T5        | Without pathogen and fungicide (Negative control) |

2.2.4. Preparation of bacterial suspension

The bacterial pathogen was cultured on the tryptone soy agar media for 24 hours and then used as an inoculum starter for preparation of bacterial suspension for the rain shelter study. For the standard curve, the bacterial suspension of BHR pathogen in sterile Luria-Bertani broth was diluted with different concentration of $10^2$, $10^4$, $10^8$, $10^{16}$ and the absorbance readings were measured. From this standard curve, the bacteria suspension was adjusted to $10^8$ cfu ml$^{-1}$ for rain shelter study. The 100 mL of the suspension was used for each experimental unit at the rain shelter as suggested by Sutton (2011).

2.2.5. Inoculation of sucker MD2 pineapple

The bacterial inoculum was introduced to the experimental units by pouring 100 mL of bacterial suspension at the centre of pineapple suckers. This wound at the centre of the suckers was served as an entry route for the bacteria to enter host tissues. This method was used to enhance the fast infection. The initial disease symptoms were observed after 7 days of inoculation; therefore, the disease progression was observed and recorded at 7 days after inoculation and up to 42 days. The disease assessment was performed after week-1 and the data of disease incidence (%) were recorded every week-1 until week-6.

2.2.6. Disease incidence (%) index

In this study, the disease incidence refers to the total number of leaf with the BHR symptoms and can be pulled out from the plant divided with the total number of observed leaf of plant. The data were calculated based on how many of the infected leaves can be pulled out after the symptoms appeared. Disease incidence is a percentage of diseased leaves per plant in the treatments. The data were calculated based on the formula below:

$$\text{Disease incidence (\%)} = \frac{a}{b} \times 100$$

Whereas $a =$ Number of leaf pull out from plant, $b =$ total number of observed leaf

2.2.7. Data analysis

Means of the disease incidence(%) for 6 weeks were calculated using one-way ANOVA in SPSS V25 with the significant differences among the means ($P = 0.05$) for each of the treatments.
2.2.8. Dipping and spraying technique
There were five treatments for this experiment and the treatments were same the previous treatments. Each fungicide with the exact concentration and amount as the previous experiment was sprayed to all the experimental units in the designated treatments by using a hand sprayer.

2.2.9. Preparation of bacterial suspension and inoculation on sucker MD2
The preparation of the bacterial suspension and inoculation for each treatment followed the procedures described in sections 2.2.4 and 2.2.5.

2.2.10. Disease severity index (%)
In this study, disease severity is used to measure the disease progress. Normally, the disease severity value is represented in a single number of disease severity index. The percentage of the total area is expressed from the area of a sampling unit affected by the disease. The disease severity on pineapple leaves was scored at 7 days after the inoculation based on the scale adapted from a scale used for soft rot disease on the lily plant by Lee et al. and Ramachandran et al (2015). The disease severity was determined after the initial symptoms appeared by calculating the area of infected on leaves. The data area for symptoms rotting disease were measured by using the transparent plastic grid. The data were calculated based on the formula below.

\[
\text{Disease severity index (\%)} = \frac{c}{d} \times 100
\]

Whereas \( c \) = area of plant tissue affected by BHR rot, \( d \) = total leaf area

3. Result and Discussion

3.1. Isolation of pathogen
A pure culture of these bacteria was tested with 3% KOH test, catalase test 3% H\(_2\)O\(_2\), potato tuber decay test, and mobility of bacteria under light microscope after 24 hours grown in the nutrient broth while shaken using shaker 500 rpm at 25°C. According to the results of 3% KOH, catalase test 3% H\(_2\)O\(_2\), potato tuber decay, mobility under light microscope, the bacterial isolates of D.C.4, and D.C11 were suspected as BHR pathogen (Table 2).

Table 2. Summary of the result of physiological and chemical properties of the bacterial isolates from infected pineapple leaves with symptoms of bacterial heart rot disease.

|            | 3% KOH | 3% H\(_2\)O\(_2\) | Potato tuber decay | Mobility bacteria | Morphology |
|------------|--------|------------------|-------------------|------------------|------------|
| D.C1       | +      | Slow bubble      | Rotten            | Slow moving      | Rod shape  |
| D.C4       | +      | Slow bubble      | Rotten            | Slow moving      | Rod shape  |
| D.C7       | +      | Slow bubble      | Rotten            | Slow moving      | Rod shape  |
| D.C8       | +      | Moderate bubble  | Rotten            | Slow moving      | Rod shape  |
| D.C9       | +      | Slow bubble      | Rotten            | Not moving       | Rod shape  |
| D.C11      | +      | Rapidly bubble   | Rotten            | Fast moving      | Rod shape  |
3.2. Pathogenicity test
The D.C 4 and D.C 11 isolation showed the BHR disease symptoms developed by the inoculated suckers were observed after 72 hours of the bacterial inoculation. Initial symptoms of BHR such as water-soaked, bloated wounds, and brownish changes around the heart of suckers were observed and recorded. Symptoms developed rapidly showing different blisters on the inoculation leaves. At the end of the disease, the development of the symptoms is approximately 21 days after inoculation. Further isolating pathogens from leaves show symptoms of the disease, confirming causal pathogen by physiological and biochemical tests, through Koch’s postulate.

3.3. Metabolic profile) Biolog) test
The identification was performed based on the cultural and morphological characteristics and the Biolog MicroLog Microbial Identification System® (Biolog Inc., Hayward,CA). D.C 11 was confirmed as Dickeya chrysanthemi or Erwinia chrysanthemi, a causal pathogen of BHR. The isolate of D.C 11 had a mucoid and white colour colony on NA, a rod shaped and very fast moving when observed under light microscope.

3.4. Effect of fungicides on BHR pathogen in vitro
The highest mean of bacterial colonies found on poison agar for each level of concentrations was benomyl. This result indicated that this fungicide is least effective to inhibit the bacterial pathogen. The mean of bacterial colonies grown on poison agar emended with the benomyl was between 83.00 – 60.75. In contrast, fosetyl-al effectively inhibited the bacterial colonies at 750 mg a.i/L concentration showed that the bacterial colony was still able to grow with the mean of 51.25. The lowest effective concentration that can inhibit the bacterial pathogen was at 250 mg a. i/L from the treatments of difenoconazole and mancozeb fungicides with the mean 0.00(±0.00) (Table 3).

| Types of fungicide | Concentration fungicides |
|--------------------|--------------------------|
|                    | 0mg/L | 250mg/L | 500mg/L | 750mg/L | 1000mg/L | 1500mg/L |
| Difenoconazole 25% EC | 94.00±9.41 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00±0.00 |
| Mancozeb 80% WP | 94.00±9.41 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 |
| Fosetyl-AL 80% WP | 94.00±9.41 | 98.75±18.13 | 51.25±0.500 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 |
| Benomyl 50% WP | 94.00±9.41 | 83.00±12.91 | 78.00±40.23 | 73.25±3.30 | 64.00±3.91 | 60.75±0.95 |

Note: The values are mean of four readings ± standard deviation

3.5. Disease incidence index (DI) (%)
The data were collected from week 1 until week 6. The control of the pathogen was found to have 2.20% of disease incidence from week 1. The least effective among the four treatments is benomyl fungicide with 20.29 %. It is because during week 1, benomyl treatment is the most of leaf can be pulled out from the plant.

This is followed by mancozeb with the mean 16.13%, the second lowest effective to control the pathogen of the disease. The most effective fungicide that can resist the pathogen from being infected is the difenoconazole fungicide with 13.78% which is below the other treatment. Meanwhile, for week
2, the mancozeb showing the least effective with 21.09%. It showed that the benomyl is less effective among others. The second less effective is the mancozeb with 11.50%. Then, difenoconazole is still showing the most effective result with the smallest mean 8.05%. Moreover, benomyl still has the highest mean compared to the others with 10.33% during week 4. It means that the benomyl is less effective.

It showed that the difenoconazole is an effective fungicide in controlling the pathogen. Besides, result during week 5 also showed that benomyl is less effective in controlling the pathogen with 9.90% while the most effective is the difenoconazole fungicide with 4.60%. Lastly, week 6, the difenoconazole also has the same mean during week 5, 4.60%. It means that the difenoconazole is the most effective as compared to the others.

The benomyl is still the highest mean among others with 8.38% by showing that it is less effective among all the others. In addition, based on the mean from week 1 until week 6, it showed that the benomyl fungicide is the least effective among other fungicides. Meanwhile, the difenoconazole was the most effective in controlling pathogen infection along the 6 weeks (Table 4).

Table 4. Disease incidence (DI) (%) recorded in four treatments from week-1 to week-6. There are significant differences among the means (P<0.05) for each of the treatments.

| Fungicide Treatment | Week 1      | Week 2      | Week 3      | Week 4      | Week 5      | Week 6      |
|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Difenoconazole      | 13.78±1.55d | 9.18±2.09d  | 8.05±1.54d  | 4.60±0.80d  | 4.60±0.78d  | 4.60±0.78c  |
| Mancozeb            | 16.13±4.39c | 21.09±4.55b | 11.50±3.86c | 7.65±2.99c  | 6.05±2.07c  | 5.62±1.90b  |
| Benomyl             | 20.29±2.82b | 16.25±2.00c | 13.72±5.18c | 10.33±2.51a | 9.90±2.07a  | 8.38±2.89a  |
| Pathogen alone      | 2.20±4.66a  | 7.73±9.35a  | 8.70±10.18a | 21.74±11.64b| 25.36±15.14b| 34.27±17.01d|

Note: Each value is representing the mean ± standard deviation of ten replications. The different alphabet between treatments indicated the significant different at P < 0.05.

3.6 Disease severity index (DSI) (%)
Each week, starting from week 1 until week 6, the benomyl fungicide showed the highest mean among the other fungicides which means it is less effective in controlling the pathogen of the disease. The mean of benomyl for week 1 is 8.25% of the leaf affected by BHR rot. Meanwhile, the difenoconazole showed 0.00% mean in week 1, which means it is the most effective in controlling the pathogen. During week 2, the mean of benomyl is 36.59 % and the mean until week 6 showing the increment for every week. For week 3, the mean of benomyl is 58.70%. Then, for week 4, the mean is 70.44% and the mean for week 5 is 81.06 %. Lastly, for week 6, the mean of benomyl is 89.81%.
Table 5. Disease severity index (DSI) (%) recorded in four treatments from week-1 to Week-6. There are significant differences among the means (P<0.05) for each of the treatments.

| FUNGICIDE TREATMENT | WEEK 1  | WEEK 2  | WEEK 3  | WEEK 4  | WEEK 5  | WEEK 6  |
|---------------------|---------|---------|---------|---------|---------|---------|
| difenoconazole + pathogen | 0.00±0.00d | 0.00±0.00d | 0.00±0.00d | 0.00±0.00d | 0.00±0.00d | 0.00±0.00c |
| Mancozeb + pathogen | 27.76±9.1d | 42.37±10.4d | 50.06±12.5d | 57.07±13.5d | 63.71±15.2d | 63.71±15.2d |
| Benomyl + pathogen | 36.59±6.8d | 36.59±6.8d | 36.59±6.8d | 36.59±6.8d | 36.59±6.8d | 36.59±6.8d |
| pathogen alone | 6.28±1.09a | 7a | 9.23±5.44a | 69.92±6.00b | 85.97±4.64b | 96.97±1.65d |

Note: Each value is representing the mean ± standard deviation of ten replications. The different alphabet between treatments indicated the significant different at P < 0.05.

Meanwhile, the findings suggested that the most effective control of pathogenic bacteria are mancozeb and difenoconazole. Based on the result of the in-vitro test, the finding showed that the fungicides difenoconazole and mancozeb effectively control bacteria Dickeya zeae syn. Erwinia chrysanthemi. For example, other study showed that the fungicides Nativo 75 WG followed by SCORE 250 EC could control the bacterial leaf blight disease in rice. There are two types of method used for this experiment which are dipping and dipping and spraying. The dipping method is known as a prevention method. While for combination of dipping and spraying method is known as a curative method. In this experiment, the mean of these two types of method is compared to evaluate the effectiveness of the selected chemical fungicides to control the disease pathogen BHR. The means of the two methods for each of the fungicides have different impacts toward the pathogen of the BHR disease.

Even if the spraying method was applied after the early symptoms appear, the infection can still reach outbreak stage. Otherwise, by comparing the means of the two method applications, this mancozeb also can control the disease but in very slow reactions in both methods, ineffectively and unrapidly. It can recover the rotting but is slower than difenoconazole. The difenoconazole is the most effective in controlling the pathogen of the disease.

When the mean of both methods is compared, the difenoconazole gives the best result for combination of dipping and spraying method. This is because after applying this method the symptoms are still rotting but in small area. Likewise, during the dipping method, the symptom of disease still appears and the leaf can still be pulled out from the plants. The best fungicide is difenoconazole by applying the combination of dipping and spraying method.

This is because there are many experienced growers in the field who also use an efficient method in applying fungicide such as spraying. The difenoconazole might reduce the infection of Dickeya. The difenoconazole fungicide by combination of dipping and spraying method might give best results but need to be re-evaluated at the fields for clarification to apply this fungicide with the combination method of dipping and spraying.

References
[1] Aeny T., SuharjoR., Ginting C., Hapsoro D., and Niswati A. (2020). Characterization and host range assessment of Dickeya zeae associated with pineapple soft rot disease in East Lampung, Indonesia. *Biodiversitas Journal of Biological Diversity, 21*(2).
[2] Brady C. L., Cleenwerck I., Denman S., Venter S. N., Rodriguez-Palen- zuela P., Coutinho T. A., and DeVo s P. (2012). Proposal to reclassify Brenneria quercina (Hildebrand and Schroth 1967) Hauben et al. 1999 into a new genus, Lonsdalea gen. nov., as Lonsdalea
quercina comb. nov., descriptions of Lonsdalea quercina subsp. quercina comb. nov., Lonsdalea quercina subsp. iberica subsp. nov. and Lonsdalea quercina subsp. britannica subsp. nov. re-emendation of the description of the genus Brenneria, re-classification of Dickeya dieffenbachiae as Dickeya dadantii subsp. dieffenbachiae comb. nov., and emendation of the description of Dickeya dadantii. Int. J. Syst. Evol. Microbiol. 62:1592-1602.

[3] Bollen GJ: (1971). Resistance to benomyl and some chemically related compounds in strains of Penicillium species. Netherlands Journal of Plant Pathology 77:187-193.

[4] Bin C., Tian Y., Zhao Y., Wang J., Xu G., Xiang L., and Hu B. S. (2020). Bleeding canker of pears caused by Dickeya fangzhongdai: Symptoms, etiology and biology. Journal of Integrative Agriculture, 19(4), 889-897.

[5] DOA. (2017). Fruits crop statistic.

[6] DOA. (2018). “Statistik tanaman buah-buahan fruit crops statistic Malaysia 2018”.

[7] Elslahi R. H., Osman A. G., Sherif A. M., and Elhussein A. A. (2014). Comparative study of the fungicide benomyl toxicity on some plant growth promoting bacteria and some fungi in pure cultures. Interdisciplinary Toxicology, 7(1), 12–16. https://doi.org/10.2478/intox-2014-0002.

[8] Gullino M. L., Federico, Tinivella, and Garibaldi A. (2010). History and role of mancozeb in disease management. Plant Disease, 94(9), 1076–1087.

[9] Hossain M. F. (2016). World pineapple production: An overview. African Journal of Food, Agriculture, Nutrition and Development, 16(4), 11443–11456. https://doi.org/10.18697/ajfand.76.15620.

[10] Hugouvieux-Cotte-Pattat, N., Condemine, G., Gueguen, E., and Shevchik, V. E. (2020). Dickeya plant pathogens. eLS, 1-10.

[11] Johnston A., (1957). Bacterial heart rot of the pineapple. Malay. Agric. J. 40:2-8.

[12] Jaji K., (2016). Value chain analysis and market factors of pineapple (Ananas comosus L. merr.) production in Johor, Malaysia.

[13] Kaneshiro W. S., Burger M., Vine B. G., De Silva A. S., & Alvarez A. M., (2008). Characterization of Erwinia chrysanthemi from a bacterial heart rot of pineapple outbreak in Hawaii. Plant Disease, 92(10), 1444–1450. https://doi.org/10.1094/PDIS-92-10-1444.

[14] Lim W. H., and Lowings P. H. (1979). Pineapple fruit collapse in peninsular Malaysia: symptoms and varietal susceptibility. Plant Dis. Rep. 63:170-174.

[15] Lee Y.A., Chen K.P., Hsu Y.W. (2006). Characterization of Erwinia chrysanthemi, the soft-rot pathogen of white-flowered calla lily, based on pathogenicity and PCRRFLP and PFGE analyses. Plant Pathology 55(4): 530–536.

[16] Mohammad, Prabhhat, Durgeshhal C., Sahroj Khan S. A., & Aaditya Prasad Y. (2019). Antifungal activity of three different ethanolic extract against isolates from diseased rice plant. Journal of Analytical Techniques and Research, 01(01), 47–63. https://doi.org/10.26502/jatri.007.

[17] Parkinson N, DeVos P, Pirhonen M, Elphinstone J. (2014.) Dickeya aquatica sp. nov., isolated from waterways. Intl J Syst Evol Microbiol 64: 2264-2266.

[18] Paragannavar S. B. (2017). Innovative Methods For Management Of Buckeye Rot Of Tomato (Solanum lycopersicum L.). Virginia Polytechnic Institute and State University,. Dr Yashwant Singh Parmar University Of Horticulture And Forestry Nauni, Solan (Hp) – 173230 India.

[19] Qudsia H., Akhter M., Riaz A., Haider Z., & Mahmood A. (2017). Comparative efficacy of different chemical treatments for paddy blast, brown leaf spot and bacterial leaf blight diseases in rice (Oryza sativa L.). Applied Microbiology: Open Access, 03(03). https://doi.org/10.4172/2471-9315.1000138.

[20] Ramachandran K., Manaf U. A., & Zakaria, L. (2015). Molecular characterization and pathogenicity of Erwinia spp. associated with pineapple (Ananas comosus (L.) Merr.) and
papaya (Carica papaya L.), 55(4). https://doi.org/10.1515/jppr-2015-0053.
[21] Johnston A. (1957). Bacterial heart rot of the pineapple. Malay. Agric. J. 40:2-8.
[22] Kole, Roy, Panja, & Worede, (2019). Use of pesticides in agriculture and emergence of resistant pests. Indian Journal of Animal Health, 58(2-SPL), 53. https://doi.org/10.36062/ijah.58.2spl.2019.53-70.
[23] Rohrbach K., & Schenck S. (1985). Control of pineapple heart rot, caused by Phytophthora parasitica and P. cinnamomi, with metalaxyl, fosetyl Al, and phosphorous acid. Plant Disease, 69(4), 320–323.
[24] Sariah M. (1989). Detection of benomyl resistance in the Anthracnose pathogen, Colletotrichum capsici. Journal of Islamic Academy of Sciences 2, 2:3, 168–171.
[25] Sindhu, G., & Joy. (2012). Diseases of pineapple (Ananas comosus): Pathogen, symptoms, infection, spread & management, (August), 493–510.
[26] Samson, R., Legendre, J. B., Christen, R., Fischer-Le Saux, M., Achouak, W., and Gardan, L.(2005). Transfer of Pectobacterium chrysanthemi (Burkholder et al.1953) Brenner et al. 1973 and Brenneria parasitica to the genus Dickeya gen. nov. as Dickeya chrysanthemi comb. nov. and Dickeya paradisiaca comb. Nov and delineations of four novel species, Dickeya dadantii sp. nov., Dickeya dianthicola sp. nov., Dickeya dieffenbachiae sp. nov. and Dickeya zeae sp. nov. Int. J. Syst. Evol. Microbiol. 55:1415-1427.
[27] Sutton, S. (2011). Determination of inoculums for microbiological testing. Vol 15 number 3.
[28] Tian Y. L, Zhao Y. Q, Yuan X. L, Yi J. P, Fan J. Q, Xu Z. G, Hu B. S, De Boer S. H, Li X. (2016). Dickeya fangzhongdai sp. nov., a plant-pathogenic bacterium isolated from pear trees (Pyrus pyrifolia). International Journal of Systematic and Evolutionary Microbiology, 66, 2831–2835.
[29] Van der Wolf, J. M., Nijhuis, E. H., Kowalewska, M. J., Saddler, G. S., Parkinson, N., Elphinstone, J. G., Pritchard, L., Toth, I. K., Lojkowska, E., Potrykus, M., Waleron, M., de Vos, P., Cleenwerck, I., Pirhonen, M., Gar- lant, L., Hélias, V., Pothier, J. F., Pflüger, V., Duffy, B., Tsror, L., and Manulis, S. (2013). Dickeya solani sp. nov., a pectinolytic plant pathogenic bacterium isolated from potato(Solanum tuberosum). Int. J. Syst. Evol. Microbiol. Online publication.doi:10.1099/ijs.0.052944-0.