The Mold Causing Agent of Rotten Snake Fruit (*Salacca zalacca* (gaertn.) from Traditional Fruit Markets

R Z Ahmad[1]*, D A Setyabudi[2] and N F Wulandari[3]

[1]Indonesian Research Center for Veterinary Science, Jl. R.E. Martadinata 30, Bogor, West Java, 16114 Indonesia
[2]Indonesian Center for Agricultural Postharvest Research and Development, Jl. Tentara Pelajar Cimanggu no:12. Ciwaringin, Bogor, West Java, 16124 Indonesia
[3]Research Center for Biology, Indonesian Institute of Sciences (LIPI) Jl Raya Bogor, KM 46, Cibinong Science Centre, Cibinong, West Java, 16911, Indonesia

*E-mail: rizamiko@yahoo.co.id

Abstract. The Rotten fruits cause of great losses. The molds and other contaminant microbes are the causing agent of rotten fruits. Snake fruit (*Salacca zalacca* (Gaernth) rotten fungi were found in the garden, market or during transportation to the market. The purpose of the experiment is to study the molds in rotten fruits at the three traditional fruit markets, Bogor, Depok and Jakarta. The mold from 122 fleshy up rotten snake fruits were isolated and identified. Colonies were grown for 3-7 days, at 25-30 ^{o}C on Potato Dextrose Agar (PDA) using direct isolation methods. Growth rate were observed every day. Molecular techniques were applied to confirm species identification. Morphology characteristics and DNA sequencing results showed that the isolates were *Thielaviopsis paradoxa*, (98.3%) and *Thielaviopsis ethacetica* (1.7%). The molds found in snake fruit from the garden and from the market were similar. *Thielaviopsis paradoxa,* is the main causes of snake fruit rotten agent in the fruit traditional markets and the initial occurrence of pollution is in the garden.
Keywords: rotten, snake fruit, *Thielaviopsis paradoxa*. *Thielaviopsis ethacetica*

1. Introduction

Snake fruit (*Salacca zalacca* (Gaerntn)) including Palmaceae is a native commodity of Indonesia. *In Indonesia salak is snake fruit.* This fruit has been included as a prime commodity because of its high potential to be marketed domestically, and developed as an export commodity. Thus, the fruit of salak has potential for agribusiness and agroindustry. Snake fruit is a tropical fruit that has the opportunity to contribute to the unfilled world market. Snake fruit has higher antioxidant activity compared with various types of tropical fruits such as avocado, apple, orange, kiwi, mangosteen, mango, melon, pineapple, banana, rambutan and watermelon [1].

In Indonesia snake fruit is not only sold in supermarkets, but also sold in many traditional markets as one of the original Indonesian fruit favored by the people of Indonesia. The snake fruit flavours is chic, sour and sweet. There are several varieties of snake fruit already known to some people of Indonesia, one

is snake fruit pondoh. This salak has a lucrative agribusiness opportunity in the future in line with the increasing demand for consumption of fruits at home and abroad [2]. While for snake fruit pondoh export commodities offer good potency especially when classified as organic fruit [3].

However, snake fruit has the nature of easily damaged and short-lived. Generally the salak fruit can only survive stored approximately 7 days at a temperature of 25–32^{o}C. This is because of the salak fruit has a moisture content of up to 78% and carbohydrate content of 21%, causing of more rotten salak if stored at a temperature of 25–32^{o}C [4]. In addition, microorganism activity including pollutant plays a role in accelerating the decay of fruit, especially in traditional markets, because of in generally snake fruits stored ranges from 25–30^{o}C. Snake fruit in the market easily contaminated by microorganisms. Pollution can occur in the garden during harvest, on the way, and time before purchases by the consumers. The Snake fruit is possible to be consumed if fresh in appearance, hard texture, the aroma is typical of snake fruit, sweet taste, the color of the flesh is still white and not overgrown with mold (kapang) (figure 1a)[5].

It is also known that fungi or molds are the most common pathogenic microbes in postharvest fruits, the level of invasion which influenced by the level of ripening of fruits and environmental conditions [6]. This preliminary research aimed to isolate and identify fungal contaminant in snake fruit of Traditional market of Bogor, Depok and Jakarta.

2. Materials and methods

The total of 121 samples of rotting pondoh snakefruit (figure 1b) were collected from traditional markets of Bogor, Depok and Jakarta. The samples were isolated and identified in the laboratory of Mycology Indonesian Research Center for Veterinary Science (IRCVS). The samples were isolated [7], inoculated on Potato Dektrosa Agar (PDA) medium and incubated at room temperature 25–30^{o}C, for 3-7 days. Morphology characteristics (conidia and hyphae) were examined by using microscopes and cultural characteristics was conducted in triplo. The molds were identified followed [8,9]. Subsequently the molds that have been identified based on macroscopic and microscopic morphology are reinforced by molecular approaches through identification by molecular DNA identification at molecular laboratory, InaCC, Research Center for Biology, LIPI.

Figure 1a. Fresh Snakefruit

Figure 1b. Rotten Snakefruit

.

2.1. Molecular phylogeny

DNA extraction was done using the Plant Genomic DNA Kit Tiangen (No CAT DP305) according to manufacturer's protocol. The primers ITS1 and ITS4 [10] were used to amplify the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA operon, including the 3' end of the 18S rRNA, the first

internal transcribed spacer region, the 5.8S rRNA gene; the second internal transcribed spacer region and the 5' end of the 28S rRNA gene. To resolve taxa in the *T. paradoxa* the primers ACT-512F and ACT-783R [11] were used to amplify part of the actin gene (ACT). Amplification conditions followed [12,13] Amplicons were sequenced using both PCR primers with a BigDye Terminator Cycle Sequencing Kit v. 3.1 (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions, and sequences were analysed on an ABI Prism 3700 DNA Sequencer (Perkin-Elmer, Norwalk, Foster City, CA, USA). The ITS and Actin sequences determined in this study were compared to those in the GenBank databases using the nucleotide Basic Local Alignment Search Tool (BLASTn) [14].

3. Results

Of the 121 rotten fruits samples were found 119 molds of *Thieviolopsis paradoxa* (98.4%) (figures 3a, b) and 2 molds of *Thielaviopsis ethacetica* (1.6%) (figure 4a, b). In the third market found *Thieviolopsis paradoxa* and only on the market found in Depok Thielaviopsis ethacetica . With the composition of 20 fruit samples from Jakarta, 41 from Depok and 60 from Bogor (table 1) and (figure 2).

Table 1. Isolates of the findings and the origin of snake fruit isolated and identified from traditional markets

The origin of snake fruit	Number	The name of taxa	The number of isolates	Percentage (%)
Bogor	60	*Thieviolopsis paradoxa*	60	49.6
Depok	41	*Thieviolopsis paradoxa*	39	32.2
		Thielaviopsis ethacetica	2	1.7
Jakarta	20	*Thieviolopsis paradoxa*	20	16.5

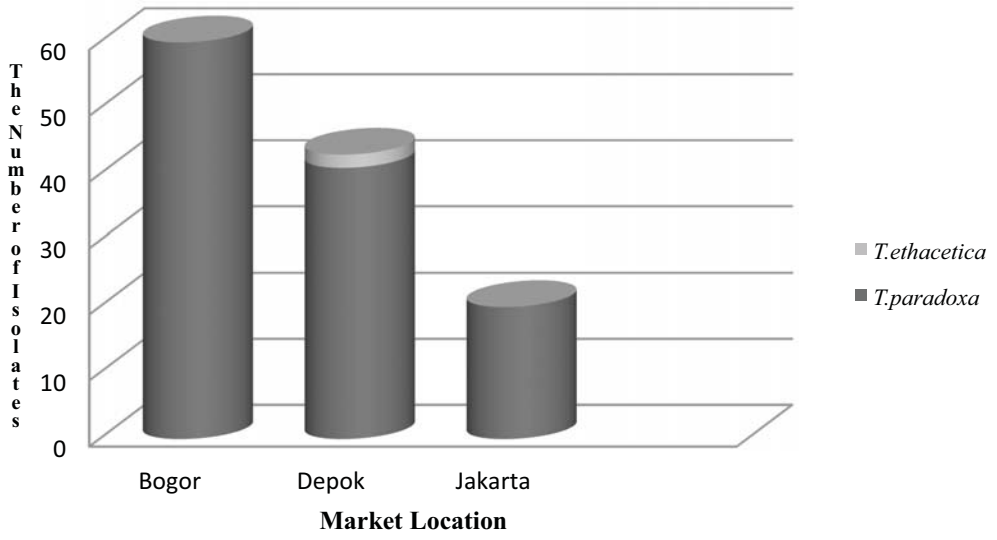

Figure 2. Isolate of fungi found on rotting snake fruits in the market

Figure 3a. *Thieviolopsis. paradoxa* in Potato Dextrosa Agar (PDA) medium, incubated in temp 25-30 ^{o}C for 7 days.

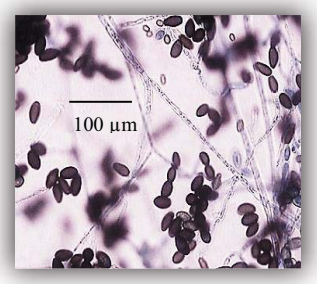

Figure 3b. *Thieviolopsis paradoxa.* Lactofenol cotton blue staining, magnification 10x40.

.

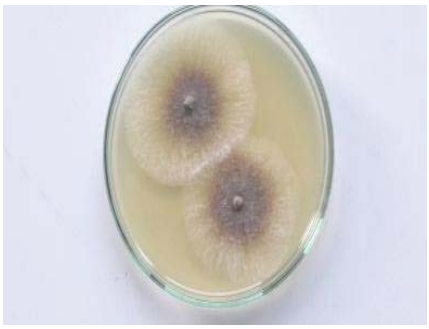

Figure 4a. *Bahusakala olivaceonigra*, or *Thielaviopsis ethacetica* in Potato Dextrosa Agar (PDA) medium, incubated in temp 25-30^{o}C for 7 days .

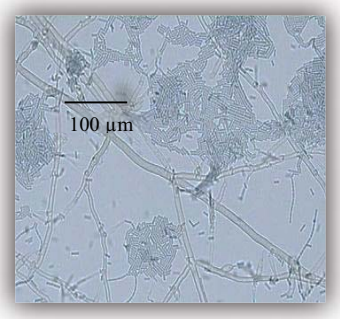

Figure 4b. *Bahusakala olivaceonigra*. or *Thielaviopsis ethacetica* .Lactofenol cotton blue staining, magnification 10x40

We are carrying a molecular work. The DNA was send for sequencing. Once we get the sequence, we compared to those in the GenBank databases using the nucleotide Basic Local Alignment Search Tool (BLASTn). After DNA sequencing the results obtained are as follows (figure 5a, 5b).

```
          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
5        15       25       35       45       55
contigTpar   TCTCCGTTGG TGAACCAGCG GAGGGATCAT TATCGAGTTT TTAACTCTTA AACCATTTGT
....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
65       75       85       95       105      115
contigTpar   GAACTTACCT TCTAGCTGCT TTGGCAGGTC CTTCGGGATT TGCCGGTAGC ACAAACAAAC

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
125      135      145      155      165      175
contigTpar   TCTTTATATT TCTATAGAAT TATTCATTGC TGAGTGGCAT TAACTAAATA AGTTAAAACT

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
185      195      205      215      225      235
contigTpar   TTCAACAACG GATCTCTTGG CTCTAGCATC GATGAAGAAC GCAGCGAAAT GCGATACGTA

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
245      255      265      275      285      295
contigTpar   ATGTGAATTG CAGAACTCAG TGAATCATCG AATCTTTGAA CGCACATTGC ACCTGGCAGC

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
305      315      325      335      345      355
contigTpar   ATTCTGCCAG GTATGCCTGT CCGAGCGTCA TTTCACCACT CAAGCTCTGC TTGGCGTTGG

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
365      375      385      395      405      415
contigTpar   AGGACCCGCG TTTGCGGGCC GCCGAAATGA ATCGGCTGTT ATACTTGCAG CTTCCCTGCG

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
425      435      445      455      465      475
contigTpar   TAGTAATTTT ATGTTACGCT TTGAAACTCT TGTACTACAT GCCGTTAAAC CCTCAATTTT

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
485      495      505      515      525      535
contigTpar   TTGAAAGGTT GACCTCGGAT CAGGTAGGAA TACCCGCTGA ACTTAAGCAT ATCAATAAGC

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
545      555      565      575      585      595
contigTpar   GGAGGAAAAG AAACCAACAG GGATTGCCCT AATAACGGCG AGTGAAGCGG CAACAGCTCA

....|....| ....|....| ....|....| ....|....| ....|....| .
605      615      625      635      645
contigTpar   AATTTGAAAT CTGGCTACTT TGTAGTCCGA GTTGTAATTT GTAGAGGATG C
```

Figure 5a. The sequenced DNA of this isolate is *T.paradoxa*

```
....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
  5       15       25       35       45       55
Contig T p   GCGGAGGGAT CATTATCGAG TTTTTAACTC TTAAACCATT TGTGAACTTA CCTTCTGGCT

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
  65       75       85       95       105      115
Contig T p   GCTTTGGCAG GTCCTTCGGG ATTTGCCGGT AGCACAAACA AACTCTTTAT ATTTCTAGAG

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
  125      135      145      155      165      175
Contig T p   AATTATTCAT TGCTGAGTGG CATTAACTAA ATAAGTTAAA ACTTTCAACA ACGGATCTCT

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
  185      195      205      215      225      235
Contig T p   TGGCTCTAGC ATCGATGAAG AACGCAGCGA AATGCGATAC GTAATGTGAA TTGCAGAACT

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
  245      255      265      275      285      295
Contig T p   CAGTGAATCA TCGAATCTTT GAACGCACAT TGCACCTGGC AGCATTCTGC CAGGTATGCC

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
  305      315      325      335      345      355
Contig T p   TGTCCGAGCG TCATTTCACC ACTCAAGCTC TGCTTGGCGT TGGAGGACCC GCGTTTGCGG

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
  365      375      385      395      405      415
Contig T p   GCCGCCGAAA TGAATCGGCT GTTATACTTG CAGCTTCCCT GCGTAGTAAT TTTGTGTTAC

....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
  425      435      445      455      465      475
Contig T p   GCTTTGAAAC TCTTGTACTA CATGCCGTTA AACCCATCAA TTTTTTGAAA GGTTGACCTC

....|....| ....|....| ....|....| ...
  485      495      505
Contig T p   GGATCAGGTA GGAATACCCG CTGAACTTAA GCA
```

Figure 5b. The sequenced DNA of this isolate is *T. ethacetica*

4. Discussion

The mold of *T. paradoxa* is almost found in all rotting snakefruit. This mold is common in rotten snakefruit, so in diagnosing it in accordance with [8 and 9]. Conidia, were sometimes cylindrical, 4-14 x 2-3 µm truncate at the ends, philalidic, smooth, hyaline, becoming light brown or sometimes varying in shape, cylindrical oval or slightly ellipsoidal 4-21 x 3-6 µm, with longitudinal slit smooth or in chains.

The result of DNA sequencing in isolates that morphologically showed *T.paradoxa* was the same ie *T. paradoxa* (figure 5a). However, the identification of suspected isolates of *Bahusakala* sp morphologically with the aid of DNA sequencing showed different results, ie *T.ethacetica* (figure 5b).

The use of DNA sequences for the *Thielaviopsis* and *Bahusakala* genus is important for accurate identification of species. Because the species of the complex exhibit few morphological differences from the asexual and sexual structures, often overlap within species when observed.

Although the morphological examination as microscopic morphology are *Bahusakala sp*. *Bahusakala* sp, have thallic-conidia and main conidiophore axes disarticulate randomly. *Bahusakala* sp produces micronematous to semi-macronematous, decumbent or prostate conidiophores that form pulverous or pulvinate colonies [8]. But the affirmation of molecular diagnosis leads to *Thielaviopsis ethacetica* rarely found in horticultural crops.

The morphological description according to [15] is quite appropriate ie *T. ethacetica* mold. The mold have ascomatal bases globose, light brown, display dark as result of aleurioconidia and distinctly digitate or stellate appendages. Ascomatal necks long, tapering to apex, dark grey. Ostiolar hyphae divergent, hyaline. Ascidehiscent. Ascospores aseptate, ellipsoidal, hyaline with sheath. Conidiophores mostly hyaline, phialidic, mononematous with enteroblastic conidium ontogeny, lageniform, solitary, occasionally aggregate in synnemata. Primary conidia aseptate, cylindrical, 7–16 × 4–7 µm, hyaline. Secondary conidia aseptate, cylindrical to oblong, hyaline becoming grey, thick-walled at maturity, cylindrical to oblong, 5–10 × 2–6 µm thic. Aleurioconidia subglobose, oblong or ovoid, thick-walled, forms holoblastically, singly or in chains, grey-brown.

In connection with the above, then in the present in the examination of identification of isolates, in addition to microscopic and macroscopic morphological examination, it is necessary to perform the examination with the aid of DNA sequencing in order to get the right result and correct.

4.1. The occurrance of contamination and decay

Generally like any other fruit, the snake fruit is still carrying out the metabolic process after harvest. Metabolic reactions will lead to changes in quality, appearance and condition of the fruit. These changes are caused by water absorption, enzymatic conversion into sugar, flavor formation and release, enzymatic conversion of pectin compounds, synthesis or degradation of pigments, vitamin breakage and microbial contamination [16]. The rate of ongoing respiration can affect the shelf life of fruits and vegetables. The faster the respiration rate the shorter the shelf life. Conversely, the slower the rate of respiration, the longer the shelf life [17]. Actually snake fruit has a natural coating on the surface that will cover the pores of the fruit so as to reduce respiration and transpiration, but with the presence of contaminants on the fruit resulted in physical damage, marked changes in color, shape and texture, as well as chemical changes characterized by changes in aroma, taste and sugar content. The presence of contaminant mold and the rapid rate of respiration causing fruits in the traditional market in the survey will be rapidly decayed, so that generally snake fruit must be sold a week after delivered from the garden.

The fungi found in snake fruit in the third market research are *T. paradoxa* and *T. ethacetica.* Generally the fungi found in the snake fruit are *Chalaropsis* sp., *Thielaviopsis* sp., *Colletotrichum* sp., *Erythricium salmonicolor, Fusarium* sp. and *Aspergillus* sp., [18]. In the market in Bogor, Depok and Jakarta found *T. paradoxa* mold on 119 samples of snake fruit. Meanwhile, from the market of Depok found *T. ethacetica* on 2 samples of snakefruit. Although the snake fruit is rarely found *T. ethacetica*, but the snakefruit sampling in the market Depok found *T. ethacetica*. This mold usually grow in plants. So that the type of *T. ethacetica* sp. add type of mold that can make rotten in snake fruit. So the contamination of this mold is to further need to watch out for.

When the fruits are exported out of the country the snake fruit should be free of some molds like in Australia and New Zealand. In this country the fruits entering these two countries should be free: *Aspergillus* sp., *Cercospora* sp., *Erythricium salmonicolor, Fusarium* sp., *Lasiodiplodia* sp., *Marasmius* sp., *Myceria* sp., *Pestalotia* sp., and *T. paradoxa* [19, 20]. Apparently the results of sampling in the market found *T. paradoxa* in large numbers as much as 98%. This should get the attention of the government that the fruits from Indonesia must be free of this type of molds when it will be exported to Australia and New Zealand. The Control of molds can be done with biological control and chemicals.

T. paradoxa contamination is commonly found in snake fruit gardens. Pollution can occur when the fruit is still in the tree or after being harvested. Generally contamination can occur in leaves, flowers and fruits. Of the contaminated fungi species can be known where the pollution occurred [18]. So it can be considered that the contamination of fruits already occurred in the snake fruit garden. The control of *T. paradoxa* and other fodder in the snake fruit garden can be done with biological agents such as *Trichoderma harzianum*, as well as with traditional crops such as *Carica Papaya* [21 and 22].

5. Conclusion

Of the 121 samples of rotten snake fruit are found 119 *Thieviolopsis paradoxa* from the 3 markets and 2 *T. ethacetica* from the market Depok. Snakefruit already polluted in the garden. Molecular examination absolutely must be done for confirmation of diagnosis of fungal contamination.

Acknowledgments

Authors would like to acknowledge for all of the contributors. ICRVS staffs are thanked for technical assistant, mycology Lab Technicians, ICAPRD for snake fruit literature support and RCBIIS. Gita, molecular laboratory, RCB Biology LIPI are thanked for molecular work assistant. EcoBio science committees are thanked for the accepting the article for proceeding.

References

[1] Aralas S, Maryati M and Mohd B A F 2009 *Nutr.Food. Scienc. J*. **39**(3) 243-250
[2] Widyastuti Y E 1996 *The Recognize of Superior Fruit in Indonesia* (Jakarta: Penebar Swadaya)
[3] Sukewijaya I M, Rai I N and Mahendra M S 2009 *Asian. J. Food Ag-Ind* Special Issue S37-S43.
[4] Manurung V H, Djarkasi G S S, Langi T M and Lalujan L E 2013 Analysis the physic characteristic and chemical characteristic of Pangu Snake Fruit (*Salacca Zalacca)* with waxing while in storage place 1-9 (https://ejournal.unsrat.ac.id/index.php/cocos/article/view/2493)
[5] Suhardi, Trenggono and Santosa U 1997 *Agritech* **17**(1) 6-9
[6] Korsten L 2006 *Int. J. of Postharv. Tech and Innov* **1** 48-61
[7] Thompson J C 1969 *Techniques for the isolation of the common pathogenic fungi. Medium*. MAFF.CVL. (England: Weybridge)
[8] Ellis M B 1971 *Dematiaceous Hyphomycetes* (Kew, Surrey, England: Commonwealth Mycological Institute) pp 13, 29-31
[9] Dos-Santos AF, Inacio CA, Guedes MV, Tomaz R 2012 *Summa Phytopath Botucatu* **38**(4) 345-346
[10] White T J, Bruns T, Lee S, and Taylor J 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics *PCR Protocols*: *A Guide to Methods and Applications* (San Diego: Academic Press) pp 315-322
[11] Carbone I and Kohn L M 1999 *Mycologia* **91** 553-556
[12] Arzanlou M, Groenewald J Z, Fullerton R A, Abeln E C A, Carlier J, Zapater M F, Buddenhagen I W, Viljoen A and Crous P W 2008 *Persoonia* **20** 19-37
[13] Wulandari N F, To-Anun C, Hyde K D, Duong L M, De-Gruyter J, Meffert J P, Groenewald J Z and Crous P W 2009 *Fung. Div.* **34** 23-39
[14] Altschul S F, Gis W, Miller W, Myers E W and Lipman D J 1990 Tool. *J. Mol. Biol.* **215**(3) 403–410
[15] Went FAFC 1893 *Mededeelingen Proefstation West-Java* **5** 1–8
[16] Pantastico E B 1989 *Postharvest Physiology, Handling and Utilization of Tropical and subtropical Fruit and vegetables* (Westport Connecticut: The Avi Publishing Company Inc.) pp 906
[17] Adirahmanto K A, Hartanto R, Novita D D 2013 *J. Agri. Tech. Lampung* **2**(3) 123-132
[18] Liptan 2000 *Identification of Snake Fruit Pest (Salacca edulis, Rein.)* (Yogyakarta: IP2TP) pp 1-2
[19] AGDA (Australian Government Department of Agricultural) 2014 Final Report Risk Analysis Report for Fresh Salacca Fruit From Indonesia *Final IRA Report Salacca from Indonesia*
[20] MPI (Ministry for Primary Industrier) 2016 *Risk Management proposal: Fresh Salacca for Consumption* (Wellington. New Zealand: New Zealand Government, Plant Import, Plants, Food & Environment, MPI)
[21] Tewari D K, Srivastava R C, Katiyar N and Bihari Lal 1988 *Indian Phytopathology* **41**(3) 491-492
[22] Ziedan E H E, Farrag E S H and Sahab A F 2015 *Int. J. Agri. Tech* **11**(4) 913-923