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Fusarium Heart Rot: First Report on Pineapple in South Cotabato and Davao City, Philippines

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Abstract

Pineapple is among the major export commodities in the Philippines. A species of Fusarium was found for the first time to be consistently associated with diseased leaves collected from MD2 'Super Sweet' pineapple plantations in South Cotabato and Davao City. Test of proof of pathogenicity of the fungus following Koch's postulates and bioassay test of Fosetyl-Al products against the fungus were conducted. For the bioassay, the treatments used were the following: 1) untreated; 2) Fosetyl-Al, Brand X at 2.25 and 5g/L water; 3) Fosetyl-Al, Brand Y at 2.25 and 5g/L water; and 4) Fosetyl-Al, Brand Z at 2.25 and 5g/L water. The experiment was laid out in Completely Randomized Design with three replications at three plates per replicate. The data was analyzed using Analysis of Variance and treatment means were compared using Tukey's Honest Significant Difference. Symptoms of the disease included lesions, which later turned into brown, necrotic tissues at the base of infected pineapple leaves, while advanced symptoms showed infected tissues with dark brown to black margins, later dried up and became soft-rotted. The fungus produced cottony white aerial mycelia on the cut surface of the tissues planted on Potato Sugar Agar (PSA) medium. Mycelia were hyaline and septated. Macro-conidia were single-celled, slightly curved, and sickle-shaped with three or more septates. Pure cultures turned light purple after eight days of incubation in full strength PSA. Chlamydospores formed after two months of incubation. Based on cultural and morphological characteristics, the fungus was identified as *Fusarium* sp. Bioassay results showed that Fosetyl-Al Brand X and Z at 5.0 g/L water significantly inhibited the growth of *Fusarium* sp. infecting pineapple leaves from 97.9 to 100%, respectively, after 9 days of incubation.

Keywords: pathogenicity test, Fosetyl-Al, soil baiting, tissue planting

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Pineapple [*Ananas comosus* (L.) Merr.] is the second most important tropical fruit in terms of global production with Costa Rica as the leading producer (11% volume share) in 2017 followed by Brazil (11%), the Philippines (10%), China, India, and Thailand. In terms of global exports, Costa Rica contributed approximately 65%, while the Philippines follows as second largest exporter at 17% volume share in 2017 (Altendorf, 2019).

Pineapple is one of the major commodities of the Philippines because of its performance in international and domestic trades and its ability to be processed into various products. Pineapple production in the country increased by 0.7% between April and June 2019 with 702.25 thousand MT compared to 697.45 thousand MT produced during the second quarter of 2018. Region X (Northern Mindanao - Bukidnon and Misamis Oriental) contributed the biggest share of the total national production (50.2%) followed by Region 12 (SOCCSKSARGEN - North Cotabato and South Cotabato) at 25.9%, and Region 5 (Bicol Region) at 8.3%. Other pineapple growing regions of the country contributed 15.5% (PSA, 2019).

Among the problems in pineapple production identified by the High Value Crops Development Program (HVCDP) of the Department of Agriculture in 2016, Phytophthora disease caused by the soil-borne fungal-like chromistans (*Phytophthora spp.*) was listed among the threats to the Philippine pineapple industry. Several species of Phytophthora cause heart and root rot in pineapple, but the most common are *Phytophthora parasitica* Dastur and *P. cinnamomi* Rands (Mehrlich, 1934). Heart rot affects basal leaf tissues and may cause the fruit to rot, while root rot causes root necrosis. Both may result to significant losses in yield.

In 2018, diseased pineapple leaves of MD2 'Super Sweet' variety collected from plantations in South Cotabato and Davao City and examined at the Crops Research Laboratory of the Department of Agricultural Sciences, College of Agriculture and Related Sciences, University of Southeastern Philippines revealed the presence of a species of *Fusarium*. Symptoms on basal leaf tissues of pineapple were similar to those induced by species of *Phytophthora* causing heart rot on pineapple. Despite repeated microscopic examinations and isolations, no *Phytophthora* was observed and isolated from infected pineapple leaves and infested soils.

Since there are no published reports of Fusarium heart rot in pineapple in the Philippines, this study was conducted with the following objectives: to establish the pathogenicity of *Fusarium* sp. associated with heart rot disease of pineapple, MD2 'Super Sweet' variety; and, to test the efficacy of Fosetyl-Al products against the fungus.

Materials and Methods

Location, collection and duration

The study was conducted at the Crops Research Laboratory of the Department of Agricultural Sciences, College of Agriculture and Related Sciences, University of Southeastern Philippines, Tagum-Mabini Campus, Mabini Unit, Pindasan, Mabini, Davao de Oro from January to May 2018.

Diseased pineapple leaves and infested soils were collected from pineapple plantations in South Cotabato and Davao City. The infected leaves (2nd and 3rd young leaves) and soil samples were placed in clean cellophane bags and brought to the laboratory for isolation.

Isolation of Fusarium sp. into pure culture

Tissue planting technique. Diseased pineapple leaves were washed in running tap water to remove dirt. About 3-5 mm tissue sections were cut from the advancing margin of the lesions. Sections were surface-sterilized in 70% ethyl alcohol for about one minute, rinsed three times in sterile distilled water, then blotted dry in sterile tissue paper. Five tissue sections were planted equidistantly to previously sterilized plated Potato Sucrose Agar (PSA) medium and were incubated for three to five days at room temperature (28-30°C). Portions of mycelia radiating from the tissue sections were transferred into fresh PSA medium using a sterile transfer needle.

Baiting from infested soil. Young, healthy pineapple leaves that were detached and surface-sterilized were used to trap the fungus from naturally infested soil. Mycelial growth from the cut surface of detached pineapple leaves was extracted and transferred to plated PSA medium using heat-sterilized needles.

Pathogenicity testing of Fusarium sp.

Garden soil contained in heat-resistant plastic bags (300g/bag) was sterilized in a pressure cooker at 121°C for 1½ hours after which the 300g soil was transferred into previously sterilized plastic containers (22 x 13 cm). Cultures of *Fusarium* sp. were grown on PSA medium for 10 days. Sterile distilled water was added to each culture, scraped gently with sterile flattened needle then filtered through sterile cheesecloth. Spore concentration was determined using a hemacytometer and adjusted to 1 x 104 spores per ml. Ten ml of the fungal suspension was poured into the sterilized soil contained

in plastic containers. Sterile distilled water was provided as control. Young, healthy, detached pineapple leaves (2nd and 3rd leaves from top) were used to test the pathogenicity of *Fusarium* sp. on pineapple. Leaves were washed in running tap water to remove surface dirt, surface-sterilized in 70% alcohol, and immediately rinsed three times in sterilized water before cutting into strips four inches long. One-inch portions of the leaves were buried into the infested soil, then incubated at room temperature until symptoms and fungus mycelia appeared on the upper cut surface of the leaves.

From the artificially inoculated detached pineapple leaves, the pathogen was re-isolated using tissue planting technique on fresh PSA medium. The re-isolated organism was compared with the original isolate obtained from the naturally infected leaves.

Identification of the pathogen

The fungal isolate was compared with Quimio's Illustrated Genera and Species of Philippine Plant Pathogenic Fungi (1983). Identification was based on the morphological (conidia, mycelia and chlamydospore formation) and cultural (colony color and mycelial growth) characteristics of the fungus.

Bioassay of Fosetyl-Al Products against Fusarium* sp. *in pineapple

The experiment was laid out in Completely Randomized Design (CRD) with seven treatments and replicated three times at three plates per replication. The treatments were as follows: Fosetyl-Al, Brand X at 2.25 and 5g/L water; Fosetyl-Al, Brand Y at 2.25 and 5g/L water; and Fosetyl-Al, Brand Z at 2.25 and 5g/L water. Sterile distilled water served as control. Fosetyl-Al had been reported to be effective against *Fusarium oxysporum* f. sp. cubense Tropical Race 4, the cause of Fusarium wilt in banana. Different brands of Fosetyl-Al were used to identify a cheaper and equally effective alternative to the more expensive Brand Z.

Poisoned food technique

To test the efficacy of different Fosetyl-Al products against *Fusarium* sp., poisoned food technique described by Burgess et. al (2008) was used. Fosetyl-Al was prepared based on specified rates and added to the cooled PSA medium in sterile flasks, shaken well before poured into sterile plates at 10 ml per plate, after which amended medium was allowed to congeal. Culture discs were obtained from 10 day-old pure cultures of *Fusarium* sp. using sterilized cork

borer and were planted at the center of the treated medium. All plates were incubated under room temperature. Observations and measurement of zones of growth and computation of the percent inhibition was done at 3, 6, and 9 days of incubation.

Data Gathered (Pathogenicity test)

Symptoms of the disease on naturally infected and artificially inoculated pineapple leaves were described. Cultural characteristics (colony color, mode of growth) and morphology of *Fusarium* sp. on Potato Sucrose Agar medium were likewise described.

Bioassay

Zone of growth (mm). The zones of growth of the fungal colony were recorded by taking the average of two long and two short diameters taken at right angles using a ruler and expressed in millimetres (mm).

Growth inhibition (%). Based on the zone of growth, growth inhibition percentage was determined using the following formula:

$$\% \text{ Growth Inhibition (GI)} = (CD–TD / CD) \times 100$$

where,

CD (Control Dish) and TD (Treated Dish) were the mean growth diameters of the fungus in the control and treated plates, respectively.

Data Analysis

Data was analyzed using Analysis of Variance and treatment means were compared using Tukey's Honest Significant Difference (HSD) when variances were significant.

Results and Discussion

Symptoms of the Disease

Naturally-infected leaves showed lesions (Figure 1a & b) which later became brown rotted tissues at the base of the infected pineapple leaves. In advanced cases, margins of infected tissues became dark brown to black (Figure

1c & d) which later on dried up while advancing portions became soft-rotted tissues (Figure 1e & f).

Figure 1. Symptoms of Fusarium heart rot in pineapple: water-soaking of infected tissues (a & b); rotting of tissues (c & d); and advanced symptoms of the disease (e & f).

Pathogenicity Test

Association of the fungus with diseased pineapple leaves. Diseased tissues examined showed the presence of a species of *Fusarium*. Macro-conidia were hyaline, sickle-shaped and multicellular (a) while micro-conidia were single-celled (b).

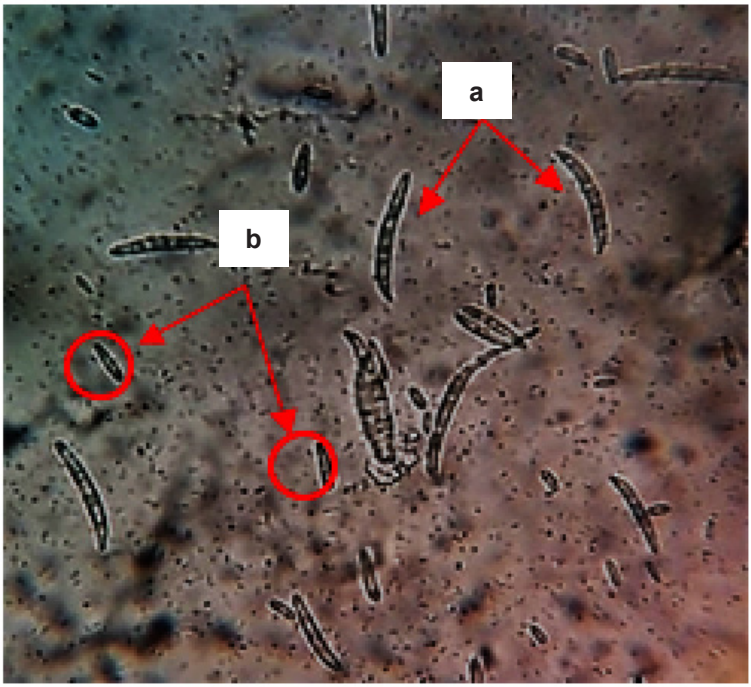


Figure 2. (a) Macro-conidia and (b) micro-conidia of *Fusarium* sp. (400x).

Isolation from infected leaves. The isolated fungus produced white aerial mycelia on diseased tissue sections planted on PSA medium as shown on Figure 3 (a). Mycelia were hyaline and septated (b). After 60 days of incubation, the fungus produced abundant chlamydospores (c) on PSA medium. Macro-conidia were slightly curved, sickle-shaped with three or more septations. Micro-conidia were non-septated similar to those observed in naturally-infected leaves (d). Pure cultures on PSA medium showed white cottony growth which turned light purple (e & f) after eight days of incubation in full strength PSA medium.

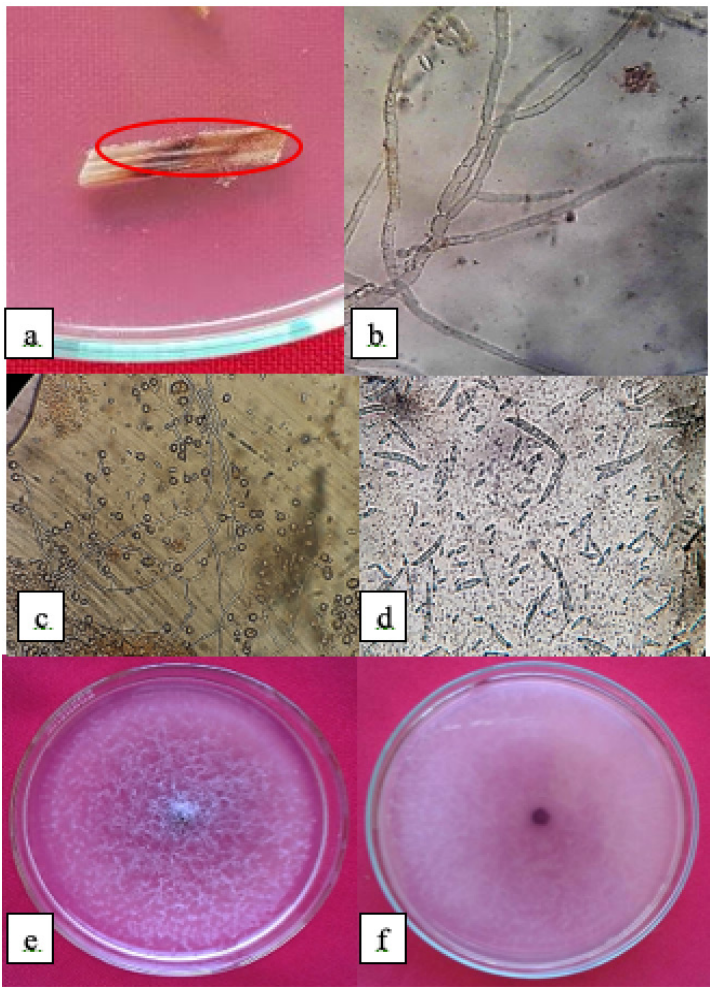


Figure 3. (a) mycelial growth on plated tissue sections; (b) hyaline and septated hyphae; (c) chlamydospores; (d) micro- and macro-conidia as seen under the microscope (400x); (e & f) pure cultures of *Fusarium* sp.

Baiting *Fusarium* sp. from infested soil. White mycelial growth of *Fusarium* sp. appeared on the cut surface of young pineapple leaves after five to seven days of baiting. Infected tissues appeared water-soaked which later on rotted as shown on Figure 4 (a). Microscopic examination revealed micro- and macro-conidia of *Fusarium* similar to those isolated from infected leaf tissues.

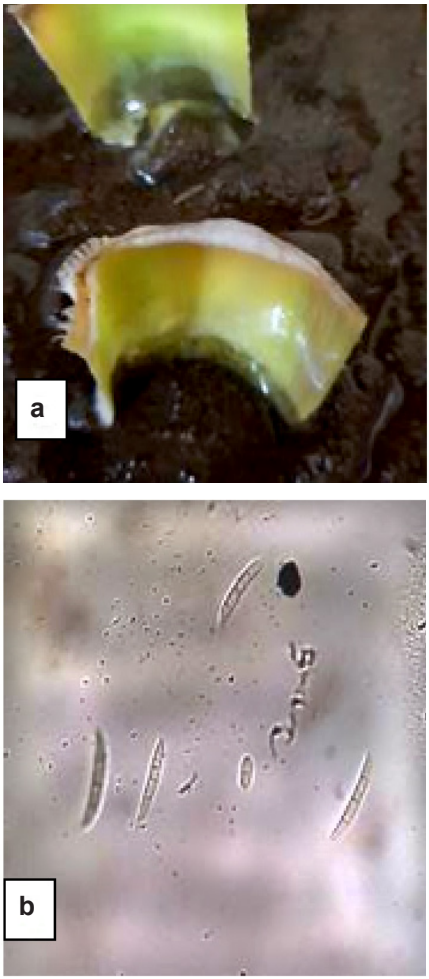


Figure. 4. (a) mycelial growth of *Fusarium* sp. on cut surface of young pineapple leaves and (b) micro- and macro-conidia of *Fusarium* sp. (400x)

Inoculation and re-isolation of *Fusarium* sp. Mycelial growth on artificially-inoculated young pineapple leaves appeared three days after inoculation of healthy young pineapple leaves suspended in fungal suspension and drenching of fungal suspension on sterilized soil. Mycelial growth that appeared was similar to the one observed earlier. The fungus was re-isolated on fresh PSA medium and microscopic examination revealed abundant micro-conidia of *Fusarium* sp. (Figure 5).

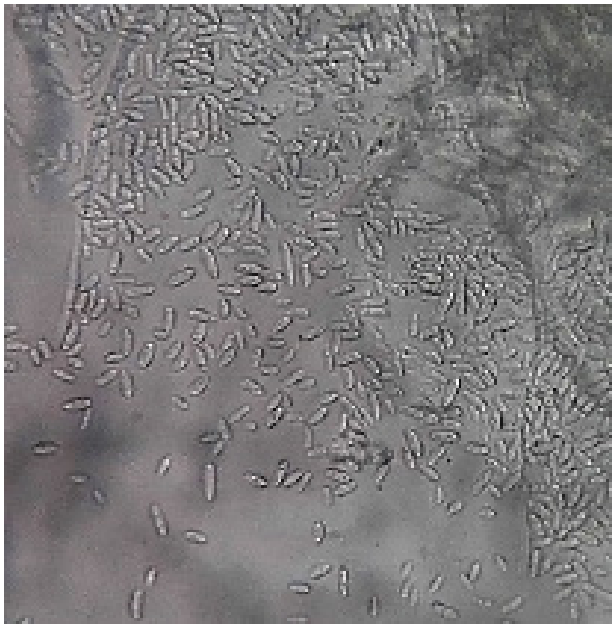


Figure 5. Mass of micro-conidia of Fusarium sp. (400x).

Identification of the fungus. Based on cultural and morphological characteristics, the fungus was identified as *Fusarium* sp. similar to the genus *Fusarium* described by Quimio (1983) in the Illustrated Genera and Species of Plant Pathogenic Fungi.

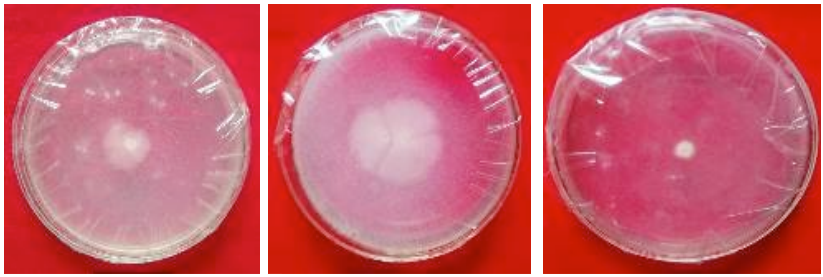
Inhibitory effects of Fosetyl-Aluminum products against Fusarium sp. in pineapple

The average zones of growth and percent growth inhibition of *Fusarium* sp. at three, six, and nine days of incubation affected by different concentrations of Fosetyl- Al products are presented in Table 1. The analysis of variance (ANOVA) revealed significant differences among treatment means. Inhibitory effects of different Fosetyl-Al products on *Fusarium* sp. are likewise shown in Figure 6.

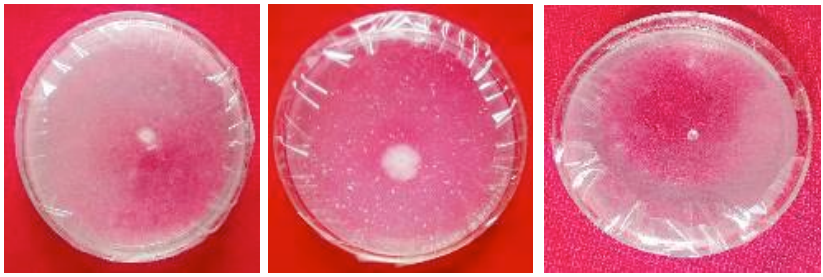
Table 1. Mean of zones of growth (ZG) and percentage growth inhibition (GI) of *Fusarium* sp. affected by different rates of Fosetyl-Al products.

CONCENTRATIONS OF FOSETYL-Al (g/L water)	DAYS OF INCUBATION					
	3**		6**		9**	
	ZG (mm)	% GI	ZG (mm)	% GI	ZG (mm)	% GI
Untreated	31.38^{d}	-	63.33^{d}	-	79.05^{c}	-
Fosetyl-Al Brand X, 2.25	0.00^{a}	100.00^{a}	11.52^{b}	81.78^{b}	20.02^{c}	74.66^{d}
Fosetyl-Al Brand X, 5.0	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	1.65^{a}	97.90^{ab}
Fosetyl-Al Brand Y, 2.25	10.83^{c}	65.25^{c}	21.91^{c}	65.40^{c}	32.44^{d}	58.94^{d}
Fosetyl-Al Brand Y, 5.0	4.49^{b}	85.70^{b}	10.27^{b}	83.77^{b}	13.60^{b}	82.79^{c}
Fosetyl-Al Brand Z, 2.25	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	4.05^{a}	94.86^{b}
Fosetyl-Al Brand Z, 5.0	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}	0.00^{a}	100.00^{a}
CV (%) =	**14.72**	**3.04**	**5.63**	**1.24**	**5.52**	**1.91**

Means having common letters are not significantly different at 1% level of significance by Tukey's HSD
Data are means of three replicates at three plates per replicate.
Percent growth inhibition (GI) was based on zone of growth (ZG) and was computed using the formula: (C-T/C)*100 where: C and T refer to the growth of *Fusarium* sp. in control and treated plates, respectively



Brand X (2.25g/L water) Brand Y (2.25g/L water) Brand Z (2.25g/L water)



Brand X (5g/L water) Brand Y (5g/L water) Brand Z (5g/L water)

Control (SDW only)

Figure 6. Inhibitory effects of different rates of Fosetyl-Al products on the growth of *Fusarium* sp. infecting pineapple on PSA medium after 10 days of incubation.

Results showed that the two rates of Fosetyl-Al products significantly reduced the colony diameter of *Fusarium* sp. at three, six, and nine days of incubation compared to the untreated control (SDW). Brands X and Z at 2.25g and 5g/L water completely inhibited the growth of the fungus at three days of incubation with 100% growth inhibition. On the 6th day, however, growth of *Fusarium* sp. was completely suppressed at a higher rate of 5g/L water for Brand X and both rates for Brand Z. Only Fosetyl-Al Brand Z at 5g/L water sustained its efficacy against *Fusarium* sp. with 100% growth inhibition (GI) up to nine days of incubation, comparable to Brand X at 5g/L water (GI= 97.9 %).

Fosetyl-Al Brand X at 5g/L water was effective against *Fusarium* sp. associated with heart rot disease in pineapple and can be used as a substitute for the more expensive Brand Z at the rate of 5g/L water. These results can be attributed to the direct effect of Fosetyl-Al's active ingredient on the fungus.

Fosetyl-Al has been previously reported against a number of biotrophs and necrotrophs. The antifungal activity appeared to be due to its phosphate compound (Fenn and Coffey, 1984) which was reported to inhibit spore germination and block mycelial growth and spore production of *Fusarium solani* (Khanzada et al. 2016).

Moreover, studies on the use of Fosetyl-Al against *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 causing Fusarium wilt in banana demonstrated the efficacy of this fungicide against the fungus (Gabio et al. 2012).

Summary and Recommendations

Based on the results of the study, it can be concluded that a species of *Fusarium* was associated with heart rot disease of pineapple. Fosetyl-Al Brand X and Z at 5g/L water significantly inhibited the growth of *Fusarium* sp. infecting pineapple leaves from 97.9 to 100%, respectively..

It is recommended that Fosetyl-Al Brand X and Z at 5g/L water be tested under greenhouse and field conditions and a comparative cost analysis be conducted. It is likewise recommended that further studies be conducted on host range to other pineapple varieties and other host plants, disease incidence and prevalence, characterization of symptoms of Phytophthora and Fusarium heart rot, and identification of the *Fusarium* sp. using the nucleotide sequence analysis.

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