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Pests and Diseases of Foxtail Millet (*Setaria italica* L. Beauv.) Cultivated in The Greenhouse

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Abstract

Greenhouse ecosystem with more stable abiotic factors could affect the population and diversity of pests and diseases found on foxtail millet (*Setaria italica* L. Beauv.) compared to their natural ecosystems. We observed and identified pests and diseases in seven genotypes of foxtail millet namely "Toraja", "ICERI 5", "ICERI 6", "Botok 4", "Botok 10", "Mauliru", and "Hambapraing". ; these activities are important for the formulation of appropriate integrated pest management techniques. Using plant samples that were at the end of their vegetative phase, we found white mycelia of *Fusarium incarnatum* (yellowish-white colonies) and *F. verticilloides* (violetish-pink colonies) covering the ear-tip of the seeds and developed rapidly leading to seed rotten symptoms in "Toraja", "ICERI 5", and "ICERI 6". The disease severity remained constant after these integrated management techniques were put in place. However, abnormalities in leaves leading to failure of panicle emergence occurred in "Botok 4", "Botok 10", "Mauliru", and "Hambapraing". These were caused by the fungi *Penicillium* sp. Identical controlling techniques were applied to this disease and resulted in low disease severity. Corn leaf aphids (*Rhophalosiphum maidis*) and rice mealybugs (*Brevenia rehi*) were recorded as main insect pests with severe attack. The aphid, *R. maidis,* colonized the stems and were associated with the sooty mold (*Capnodium* sp.), resulting in wilting. The mealybugs, *B. rehi,* colonized the flag leaves resulting in leaf rotting. Insecticide and isolating the attacked plants were used as the controlling techniques. Red-mites (*Tetranychus urticae*) were also detected as indicated by chlorotic spots on the upper part of the leaves. Acaricide was used to reduce its population. The pests and diseases found in the seven genotypes of foxtail millet are commonly known to occur in Poaceae. In general, greenhouse ecosystem for foxtail millet facilitated planting with high population with lower diversity of pests and diseases compared to the open field planting.

Keywords: pest control methods; fungal infection, pests.

Introduction

Plant pests and diseases affect food crops, causing significant losses to the growers.. Biotic stress caused by insects and pathogens affecting each other causing complexity in physiological disorders in plants. Moreover, most of pests and diseases have ability to survive over the planting season leading to an increase in attacks during the following growing season. In understanding this plant-insects-phytopathogens interaction, various techniques in integrated pest management (IPM) practices have to be applied (Franco et al., 2017)

The diversity of pests and diseases are closely related to the agroecosystems. Ecosystems in greenhouses with relatively more stable abiotic factors than in field are thought to affect the population of insect pests and diseases, insects activities and disease progression. Therefore, management of pest and diseases for the crops grown in greenhouses need modifications (Gullino et al., 2020).

Foxtail millet (*Setaria italica* L. Beauv.) is considered as one of the most common cereal grasses in Indonesia. It is known for its high nutrient values and tolerance to abiotic stress (Sharma and Niranjan, 2017; Ardie et al., 2015). As one of the oldest cultivated crops, there are pests and diseases that have been steadily associated with foxtail millet. Despite the importance of information on diversity of pests and diseases on foxtail millet, this topic has not been widely explored.

Periodic monitoring of the emergence and development of pests and diseases in foxtail millet cultivated in greenhouse is important in order to estimate the potential yield loss that could potentially occur, and come up with solutions to avoid future losses. The objectives of this study was to identify the pests and diseases of foxtail millet in the greenhouse and determine the scientific, practical, and efficient controlling methods. Specifically, the study was conducted to determine with methods on how to minimize and eventually disrupt the life cycle of pests and diseases, thereby stopping their exponential increase due to their survivability in seeds, plant debris, soil, and other media.

Material and Methods

Pest and Disease Observation

The study was conducted on January – April 2021 in Cikabayan Greenhouse, Laboratory of Plant Mycology, and Laboratory of Insect Biosystematics, Faculty of Agriculture, IPB University. The presence and progress of pests and fungal diseases were recorded on seven genotypes of foxtail millets from Indonesian Cereal Research Institute ("ICERI 5" dan "ICERI 6"), and from explorations in South Sulawesi ("Toraja"), West Nusa Tenggara ("Botok 4" and "Botok 10"), and East Nusa Tenggara ("Mauliru" and "Hambapraing"). Thirty plants of each genotypes at the end of their vegetative stage were collected.

The symptomatic plant organs were previously surface sterilized with 1% NaClO. Fungal colonies were isolated on potato dextrose agar (PDA) medium and incubated at room temperature for 48 hours. Subsequently, isolates were re-cultured in PDA medium to facilitate the optimum growth. Colonies and microscopic structures were characterized at 5 days after incubation to identify the fungi using compound microscope. Species of fungi and diseases were identified using several references, i.e., Leslie and Summerell (2006); Singh et al. (1991); and Watanabe (2002) and confirmed by plant protection specialist at the Laboratory of Plant Mycology, IPB University.

The pests were directly observed directly from the plants. The pest samples were then placed into plastic bags to be brought to the Laboratory of Insect Biosystematics for identification. Identification of specimens was conducted by observing the morphological characteristics using a stereo microscope according to identification guide obtained from various sources (Hidayat et al., 2020; Miller et al., 2014; CABI, 2019). The specimens were photographed using a Leica Microscope (M205C).

Integrated Pest Management Techniques

As a part of integrated pest management principles, disease monitoring was undertaken by measuring the disease incidence and severity every week by formulas refer to Cooke (2006). Scoring method for ear rot disease was refered to Presello et al. (2006) and modified as shown on Table 1. To anticipate the invasion of the pathogens which could lead to yield loss, integrated management techniques have been undertake, i.e. widening the inter-plants distance, reducing the watering frequency, utilizing the rhizobacteria formulation contained *Bacillus polymixa* and *Pseudomonas fluorescens* 100 ml for each plant, and applying of fungicide (Trifloxystrobin and Propiconazole). To control the pests, the infected plants were isolated along with the application of insecticide (a.i. Imidacloprid) and acaricide (a.i. Pyridaben).

Results and Discussion

Fusarium Species in Foxtail Millet

Disease symptoms in "Toraja", "ICERI 5", and "ICERI 6" genotypes were initially observed 24 days after the panicles appeared. First symptoms included the appearance of whitish mycelia on the ear tip of seeds. The whitish mycelia developed to pinkish wider colonies that covered the seeds in 5 days. Seeds covered by the pinkish colonies became rotten and empty (Figure 1). As confirmed by isolation in PDA medium, we always found two fungal colonies from this type of symptom.

Table 1. Scale used for visual disease assessment for Fusarium ear rot disease*

Scale	Mycelial coverage (%); and symptoms description
0	No symptoms
1	0 < x ≤ 3; white mycelia emerged
2	3 < x ≤ 10; white mycelia developed
3	10 < x ≤ 25; colonies developed to pinkish mycelia
4	26 < x ≤ 50; pinkish mycelia developed to clotted
5	50 < x ≤ 75; half of seeds are rotten
6	75 < x ≤ 100; all of seeds are rotten

*Scale is based on Presello et al. (2006), modified.

The two fungal colonies appeared yellowish-white and violetish-pink. These colonies grew fast in 3 days and produced aerial growth. Using microscopy, these two colonies were identified as *Fusarium* spp., both of which had ovoid microconidia with no septum. The yellowish-white colonies had three septate macroconidia. There were no macroconidia found in the violetish-pink colonies (Figure 2). The disease intensity increased in the form of expansion of colony coverage and color changes. According to Zheng et al. (2019), *Fusarium incarnatum-equiseti* species complex (FIESC) could infect the ear tip of foxtail millet panicles with pinkish colony colour. All infected plants developed severe diseases and were unable to produce any seeds.

Disease progress measurements are shown in Table 2 and Figure 3. Genotype "ICERI 6" has the lowest disease incidence (66.7%) while the genotype "Toraja" and "ICERI 5" reached above 82% in 29 days after the panicles appeared. However, this percentage significantly increased 7 days later (93.3% - 100%). We recorded that the increasing of disease severity were remains constant after the integrated management technique (Figure 3). During separate observation, we found that these colonies grew rapidly at 27°C and at >80% relative humidity, factors that are critical in designing pest control techniques.

Penicillium in Foxtail Millet

The growing period of "Botok 4", "Botok 10", "Mauliru", and "Hambapraing" genotypes were slower than the three other genotypes, so the observation for these three genotypes was undertaken later. Due to earlier disease control on these three genotypes, we found disease with low incidence and severity of <15% and <8%, respectively. However, the onset of symptoms resulting from pathogen infection was more varied. The most common symptoms were necrotic areas in twisted leaves. Moreover, there were light brown spots or blight at the tip of the leaves (Figure 4). Morphological characterization of fungi isolated from these symptoms determined the infection of *Penicillium* sp. (Figure 5). Manjula and Khrisnappa (2020) recorded the presence of *Penicillium* sp. as

Figure 1. *Fusarium* spp. during (A) initial infection, (B) severe infection and (C) rotten and empty seeds.

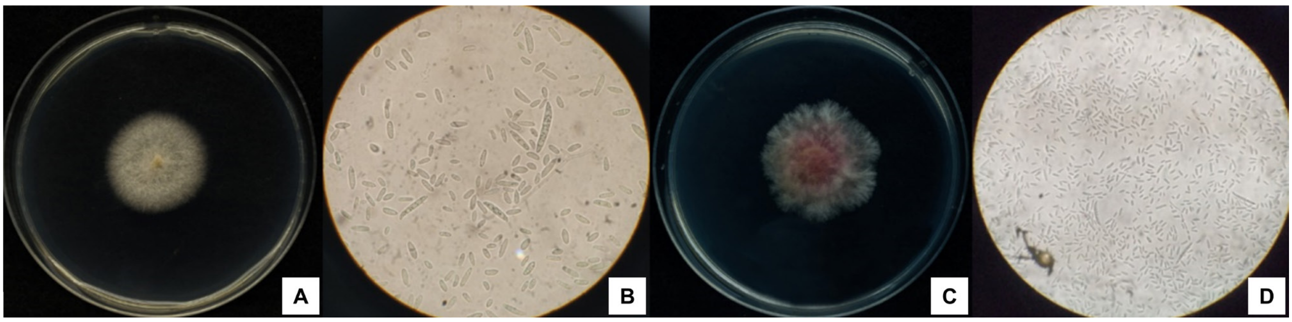

Figure 2. (A) Yellowish-white *Fusarium* spp. colony, (B) macroconidia and microconidia, (C) violetish-white *Fusarium* spp. colony, and (D) microconidia.

Table 2. Disease incidence of *Fusarium* spp. infection

Genotype	Disease incidence in n-day after panicle appeared (%)			
	29	36	42	49
"Toraja"	82.8	96.7	96.7	100.0
"ICERI 5"	86.7	100.0	100.0	100.0
"ICERI 6"	66.7	93.3	93.3	93.3

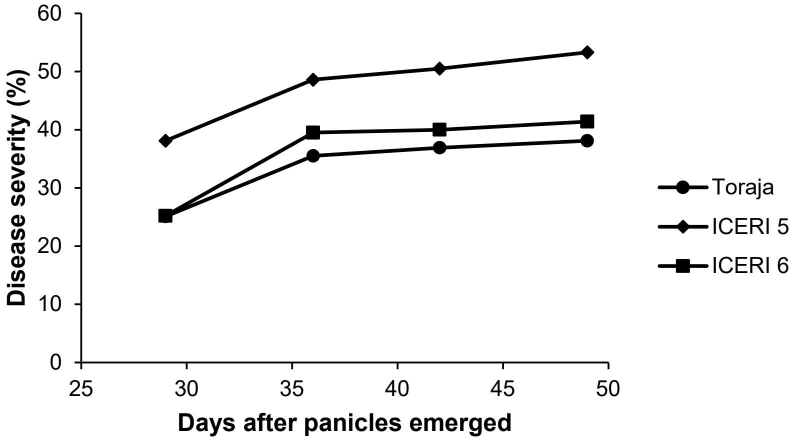

Figure 3. Disease severity of *Fusarium* spp. infection in foxtail millet

Figure 4. Symptoms found in “Botok 4”, “Botok 10”, “Mauliru”, and “Hambapraing” genotypes including (A) necrotic area in twisted leaves, (B) brown spots, and (C) leaf blight.

seed-borne fungi on foxtail millet which leads to germination failure. The occurrence of *Penicillium* sp. and symptoms associated with its presence in foxtail millet in the growing stage has not been previously reported. The diversity of symptoms, risk of yield loss, and the histopathological phenomenon of *Penicillium* infection require further investigation.

Pest Composition in Foxtail Millet

There are three species of pest found in foxtail millet, they were corn leaf aphid *Rhophalosiphum maidis* (Hemiptera: Aphididae), rice mealybug *Brevenia rehi* (Hemiptera: Pseudococcidae), and two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae). Those three pests appeared in colonies and feed by sucking plant fluids from the leaf or near the leaf sheath. The feeding behaviour of these pests results in stylet sucking (aphids and mealybugs) or chelicera (spider mites). At high population, symptoms of an attack can appear as abnormal growth on leaves and plants. Aphids and mealybugs can produce honeydew, which can stimulate sooty mold growth as a secondary pathogen. The presence of soot on the surface of leaves or plants will inhibit photosynthesis, causing the growth of plant less than optimum and produce small grains (Capinera, 2001).

Aphid *R. maidis* were frequently found in colonies on the plant (Figure 6A). The population of *R. maidis* are about 200 wingless aphids on each of foxtail millet plant. In tropical and warm areas, such as Indonesia, *R. maidis* produce new generations parthenogenetically (without mating) and it goes through four nymphal stages (Kalaisekar et al., 2010). The young nymph body is yellow in colour, with red eyes, and brownish dorsal head and siphunculi, while the late instar and adult has greenish yellow, light green, to dark green body with some dark pigmentation on head, thorax, and some appendages (antennae, siphunculi, and cauda). Furthermore, late instar nymphs and adults of *R. maidis* have elongated bodies as compared to other aphid species (Figure 6B). Symptoms of aphid attack on leaves including chlorotic yellow to brown spots on the leaf surface (Figure 6C). The presence of honeydew secretion from *R. maidis* aphid in a large population on foxtail millet may cause the development of secondary symptoms, such as sooty mold caused by *Capnodium* fungus (Figure 6D).

The corn leaf aphid, *R. maidis,* is distributed throughout the world and is known to be one of the important pests in cereal crops, especially on maize and sorghum in tropical areas. It also feeds on barley, chufa, oat, rye, millet, sudan grass, and sugarcane. Among weeds and prairie grasses, it is known to

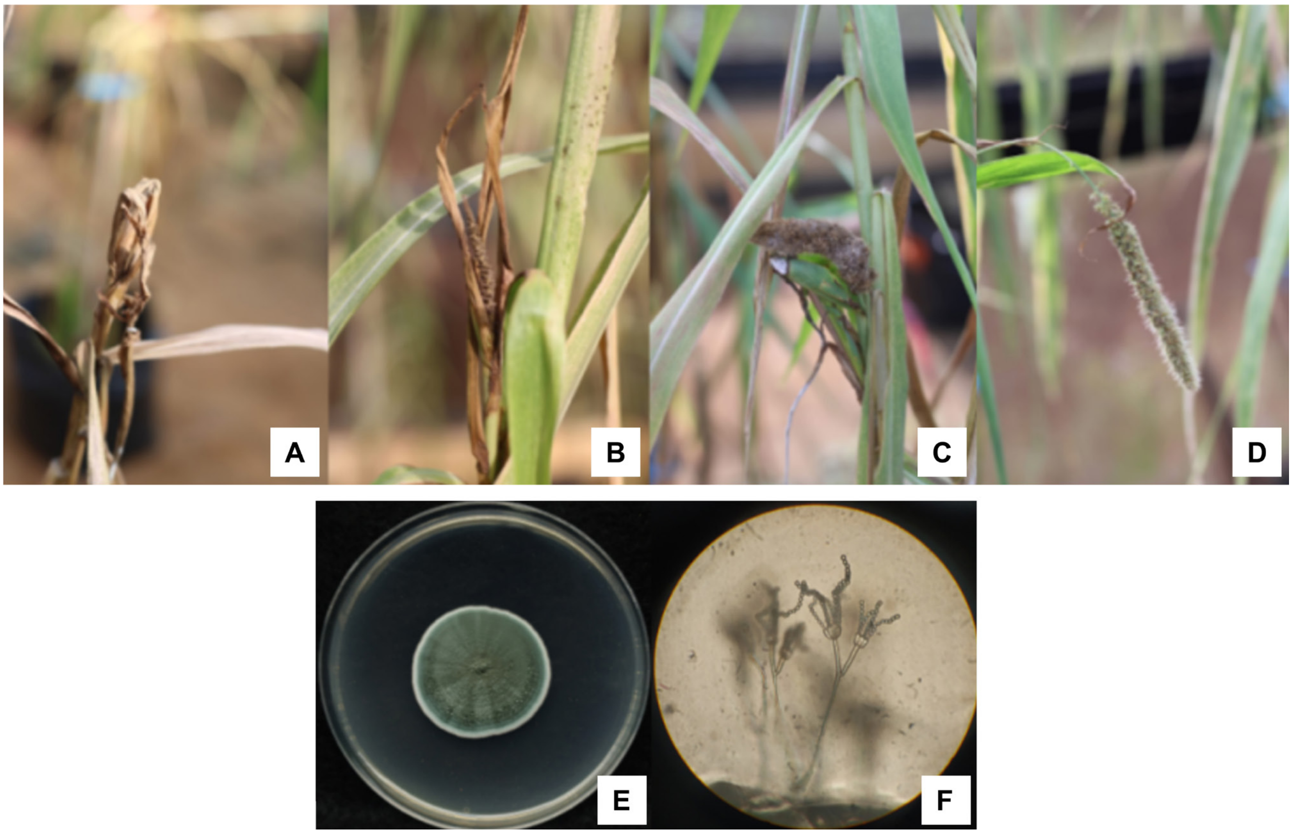

Figure 5. The variety of symptoms due to *Penicillium* sp. including (A) infection of the leaves and their effect on panicles in the form of the failure of panicle emergence, (B) rotten emerged panicles, (C) panicles malformation, (D) panicles managed to escape the disease and grow normally, (E) colony of *Penicillium* sp. on PDA, and (F) microscopic structures of *Penicillium* sp. in 1.000x optical zoom

Figure 6. (A) Presence of aphids *R. maidis* on plant, (B) nymphs and adult of *R. maidis*, (C) symptoms on the leaf, (D) and presence of sooty mold as secondary pathogens

attack barnyard grass (*Echinochloa crusgalli*), buffalo grass (*Buchloe dactyloides*), crabgrass (*Digitaria sanguinalis*), foxtail (*Setaria* spp.), grama grass (*Bouteloua* spp.), and johnson grass (*Sorghum halepense*) (Capinera, 2001). Crop plants are generally tolerant to aphids and only extremely dense infestations could cause damage and yield loss. However, if soil moisture conditions are insufficient, it is likely for aphid damage to increase and yield to decline (Capinera, 2001). In addition, *R. maidis* can transmit some plant viruses, including barley yellow dwarf virus, maize dwarf mosaic virus, maize streak virus, and maize stripe virus (Capinera 2001).

Two-spotted spider mites, T. *urticae*, is a cosmopolitan, highly polyphagous species that spreads easily via wind. It usually reaches a high population density under optimum conditions, and its presence can reduce crop yield. We found 174 mites on each plant. Among plants in the family Poaceae (grass), T. *urticae* is known to attack rice, sorghum, maize, and sweetcorn (CABI, 2019). Mites have piercing and sucking mouthparts, called chelicera, that can penetrate into plant tissues. Spider mites most commonly found on the underside of leaves and spin fine strands of webbing, hence their name (Fasulo and Denmark 2009). Two-spotted spider mites found on foxtail millet also develop webbing on the underside of the leaf (Figure 7A). The feeding process of the mites causes the leaves to turn yellow or gray in color. Chlorotic spots develop in the later stages of leaf damage (Figure 7B).

When leaves are infected with T. urticae, they suck out the juices out and the mesophyll tissues degraded, and small necrotic spots develop at each feeding site. Later, the leaves develop stippled-bleached appearance, and eventually the leaves turn to yellow, gray, or bronze in color. If the mites were not controlled, complete leaf defoliation can occur (Fasulo and Denmark, 2009). The life cycle of two-spotted spider mites include the egg, larva, two nymphal stages, and the adult (Fasulo and Denmark, 2009). The eggs are round and transparent to yellowish in color. Larva has yellow body with dark pigmentation near the head and anterior half of the body and has three pairs of legs. Nymph has red and yellowish body color with four pairs of legs. The adult body is red in color with two dark pigmented areas on each lateral side (Figure 7C).

The rice mealybug or tuttle mealybug, *Brevenia rehi*, is an insect pest of many grass species. That have cosmopolitan distribution, especially in the area where rice and sugarcane have grown (Stocks, 2013). From our observation, as many as 55 adults of *B. rehi* were found in hidden parts of the plants, such as between sheath and the stem (Figure 8A). Changes in leaf color become yellowish green, yellow, and brownish, are the symptoms of attack of *B. rehi* on leaves. In heavy infestation, the leaves will turn to brown color and and dry (Figure 8B). The body size of rice mealybug is typically small and pink in color, and usually covered by white waxes (Figure 8C). On the rice field, *Brevenia rehi* is usually found in rain-fed field areas and uncommonly found in irrigated field. Therefore, it appears in a great number during the rainy season. The main host plant of *B. rehi* include rice, maize, sorghum, sugarcane, and grasses. They are also found in other species of grass, such as *Andropogon* spp., *Cynodon* spp., *Eleusine* spp., *Eragrotis* spp., *Panicum* spp., and *Paspalum* spp. (Stocks, 2013; Plantwise, 2021).

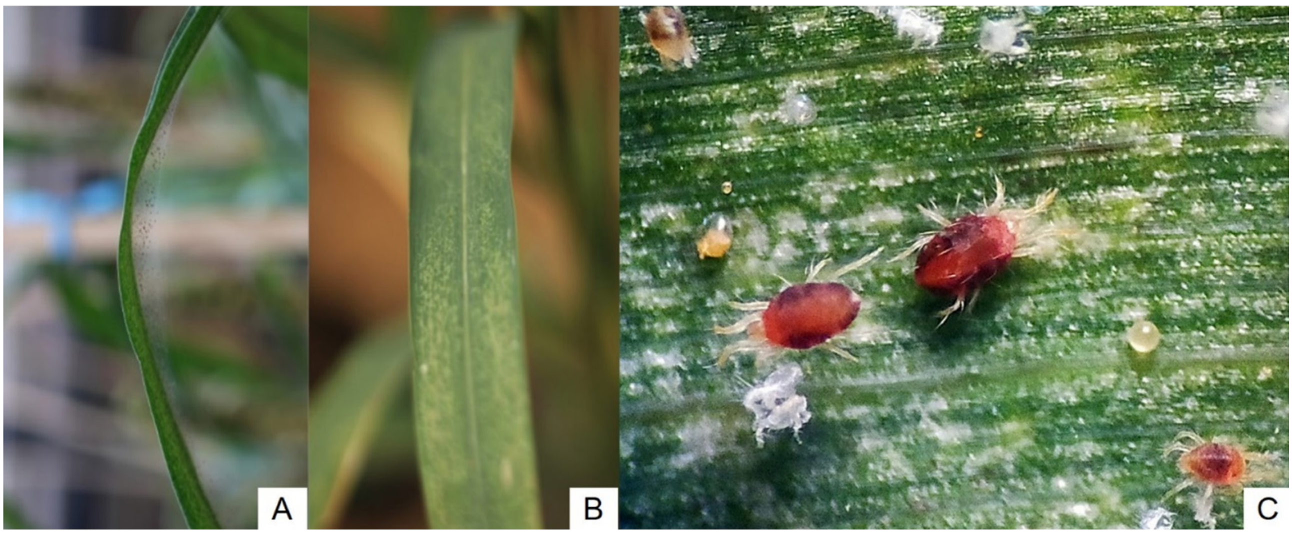

Figure 7. (A) The presence of two-spotted spider mites on foxtail millet plant, (B) symptoms on the leaf, and (C) eggs, larva, nymph, and adults of T. *urticae*

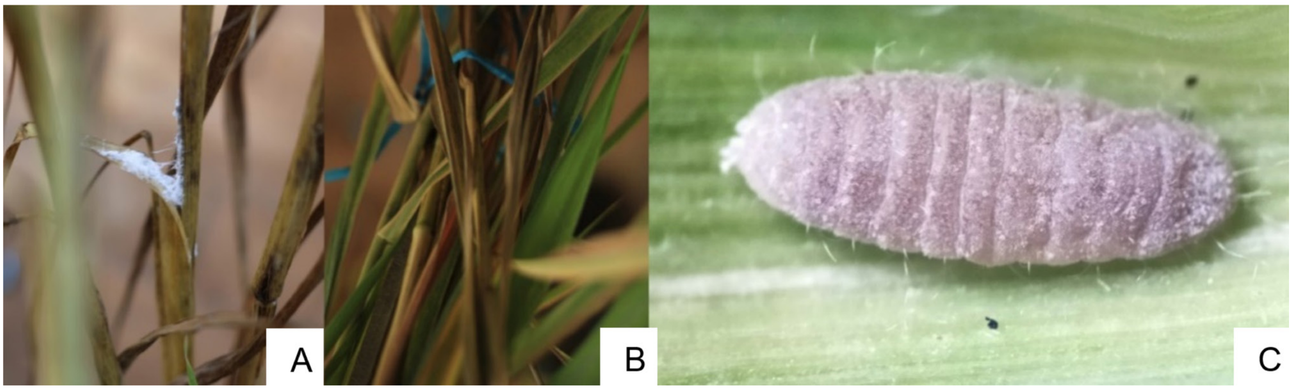

Figure 8. (A) The presence of mealybugs onfoxtail millet plants, (B) symptoms on plants, (C) mealybug on a

Conclusion

From seven known genotypes of *Setaria italica* (L. Beauv.) in Indonesia, we found the fungi *Fusarium incarnatum* and *F. verticilloides* isolated from ear-rot symptoms on the seeds of "Toraja", "ICERI 5", and "ICERI 6". At the earlier stages, *Penicillium* sp. were observed causing abnormalities on the leaves resulting in the failure of panicles to develop in genotypes "Botok 4", "Botok 10", "Mauliru", and "Hambapraing". Several pests, such as corn leaf aphids (*Rhophalosiphum maidis*), rice mealybugs (*Brevinia rehi*), and red-mites (*Tetranychus urticae*) were colonized all genotypes. Although these pests and diseases are common in Poaceae, ecosystem in the greenhouse require specific controlling techniques to suppress the infection and reduce the yield loss.

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