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THE APPLICATION OF BOTANICAL PESTICIDES TO CONTROL FUSARIUM WILT ON ASPARAGUS BEANS

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Abstract*. Triggered by a fungus of the genus Fusarium, wilting prevails in various horticultural crops in Indonesia. In asparagus beans, this pathogen can lead to decreased production. One eco-friendly controlling measure is the application of botanical pesticides made from betel leaves and neem leaves which have been extensively used to control various pathogenic fungi. This study aims to evaluate the effectiveness of plant-based pesticides made from neem and betel leaves in controlling Fusarium wilt in asparagus beans. This research was run for approximately six months, initiated at the Laboratory of Plant Protection in Universitas Borneo Tarakan, and ended in the Experimental Garden of the Faculty of Agriculture. It involved Fusarium propagation, preparation of planting media, planting asparagus beans, infecting Fusarium, and producing as well as applying botanical pesticides. It employed a one-factor randomized block design with 5 treatments, control (P0), 5% neem leaf extract per plant (P1), 10% neem leaf extract per plant (P2), 5% betel leaf extract per plant (P3) and 10% betel leaf extract per plant (P4). The covariance analysis results showed that the treatment did nothave a significant effect on the number of affected branches, the number of pods and pod weight. Even so, it can be concluded that P2 can reduce wilted branches by up to 48%, with the same concentration, and P4 can reduce wilted branches by up to 52%. In terms of the total number and the total weight of the pods, P4 achieved the best results with 77 pods, weighing 3,272 grams.*
Keywords: *azadirachta indica; piper betle; vigna sinensis*

1. Introduction

Fusarium wilt is a disease infecting various plants (Rahayu, 2015). Its symptoms are caused by Fusarium on asparagus beans in Indonesia. Fusarium also infects peppers, potatoes, tomatoes, and cucumbers worldwide (Zhao *et al*., 2018). Hartati *et al.* (2016) reported the occurrence of Fusarium in watermelon, and Mudmainah and Khatimah (2022) found Fusarium in melon. Fusarium fungus pathogens can significantly reduce agricultural production. Wegulo *et al*. (2015) contended that Fusarium head blight (FHB) is an economically devastating disease of small grain cereal crops worldwide. In horticulture, Everts *et al.* (2014) documented reduced watermelon yields due to Fusarium wilt, which has been on the rise in the United States. In Indonesia, Fusarium-infected watermelon weighs about 400 grams, lighter than similar infected fruit controlled using Gliocladium and vermicompost (Ruliyanti & Majid, 2020). Significant crop losses can cause economic losses to farmers as their income depends on their production and sales

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(Efendi, 2016).

The primary symptoms of Fusarium wilt are leaf wilting and yellowing. Sutrisni & Widodo (2012) identified these symptoms in asparagus beans. Fitriani *et al.* (2019) also reported the same symptoms on shallot plants. At a severe stage, this pathogen can cause root and stem rot, preventing plants from absorbing water and nutrients for growth. Juariyah *et al.* (2018) reported the root rot of oil palm seedlings because of Fusarium. In the same year, Sudantha and Abad (2018) reported on Fusarium stem rot on vanilla seedlings. Wahyuni *et al.* (2019) documented wilting stems and root rot on the banana.

Fusarium can be controlled through the use of synthetic pesticides and botanical pesticides. Fungicide with propinen as an active ingredient was reported to control Fusarium infection in vitro (Nurhasanah, 2020). In addition, Wahyu (2021) reported that mancozeb as an active ingredient can control fusarium wilt. These measures can address potential adverse environmental impacts of excessive chemical pesticides, such as water and soil pollution, and poisoning of non-target organisms. Kurniawan (2019) reported on environmental problems due to chemical pesticides in several areas in Indonesia. In addition, long-term use of chemical pesticides can cause resistance to pathogens. This signifies the need for an eco-friendly alternative for controlling Fusarium wilt.

Botanical pesticides are an appealing eco-friendly alternative, causing no resistance to pathogens. These are generally obtained from plant extracts that have antifungal and antibacterial activity, making them effective in controlling diseases. Indonesia is well-known for its potential botanical pesticides. In this direction, Ganul *et al.* (2021) stated that Indonesia has abundant plant biodiversity as an important element to botanical pesticides, due to its tropical climate. Some plants known for their potential as botanical pesticides include neem leaves and betel leaves.

Neem (*Azadirachta indica*) grows mainly in tropical and subtropical regions, including in Indonesia (Hashmat *et al.*, 2012). It usually grows in dry and sandy soil, but it can adapt to other soil conditions (Banerjee *et al.*, 2013). Neem has serrated leaves which consist of several smaller leaves connected to the main petiole. These small leaves have an oval or lanceolate shape with a pointed tip. The leaves are arranged in a pinnately or bipinnately compound structure (Keter, 2012). The latter means that the main petiole has small branches connected by small leaves. The pinnately compound arrangement means that the small leaves possess small branches connected to other small leaves, with varying numbers of tiny leaves. One leaf can have several pairs of small leaves. The leaf color is dark green, but it also varies depending on the amount of sun exposure and the growing conditions. Moreover, the leaves have a rough texture and a slightly wavy surface, with a few fine hairs.

The betel also grows in tropical and subtropical regions, such as Indonesia (Yolanda, 2022).

It is often found in tropical rainforests, plantations, and yards with moist soil. Betel can reproduce generatively or vegetatively. Generatively, it produces small white or yellowish-green flowers which are incorporated in small heads (Gita & Danuji, 2021). Its pollination is assisted by insects such as bees and butterflies. Vegetatively, betel propagates through stem cuttings or separating shoots. Its leaves are round to heart-shaped with flat or slightly serrated edges. However, this shape depends on genetic variation and growing conditions. The leaf base is rounded, and the tip is sharp. These leaves belong to single leaves located alternately along the vines. The leaves are usually arranged spirally around the stem, with the petiole connected to the stem. The leaf color can vary from light green to dark green depending on the age and leaf condition. Ranging from 7 to 12 cm in length, betel leaf has a soft and smooth texture with a shiny surface. The leaf fibers may also be seen from below.

Neem leaves (*Azadirachta indica*) and betel leaves (*Piper betle*) can naturally control Fusarium infection. Neem leaves contain various active compounds such as azadirachtin, nimbolin, and nimbidin (Agustin *et al*., 2016), while betel leaves contain such active compounds as flavonoids, alkaloids, and tannins which possess antifungal properties (Rahmat, 2020). Neem leaf botanical pesticide can control fusarium on tomatoes. Singh *et al*. (2015) proved that the root length of tomatoes grown on fusarium-infected land was 10 cm shorter than that treated with *Trichoderma harzianum* and neem leaf extract. Yi *et al*. (2021) concluded that the application of neem leaf extract solution promotes the growth of Cavendish banana, soil physicochemical properties, and resistance to Fusarium wilt. In-vitro tests conducted by Arsih *et al*. (2015) showed that the crude extract of betel leaf with a concentration of 0.05% to 0.35% can inhibit the growth of *Fusarium oxysporum* f.sp. vanillae which causes vanilla stem rot on Potato Dextrose Agar (PDA). Arsih *et al*. (2015) explained that the use of betel leaf extract with a concentration of 0.25% effectively suppressed the growth of *Fusarium oxysporum* f.sp. lycopersici, with reported inhibition reaching 68.89% in tomatoes.

Neem leaves and betel leaves are widely used as raw materials for botanical pesticides to control various plant diseases, one of which is Fusarium, a broad-spectrum fungal pathogen. These botanical pesticides have been reported in protecting many horticultural crops such as tomatoes and bananas from Fusarium. Following this line of inquiry, this study aims to further the investigation of botanical pesticides made from neem leaves and betel leaves in controlling Fusarium in asparagus beans.

2. Methods

2.1. Research Site

This research began with the multiplication of Fusarium isolates in the Laboratory of Plant

Protection, at Universitas Borneo Tarakan, to test botanical pesticide against fusarium wilt on asparagus beans. The experiment was performed in the Experimental Garden of the Faculty of Agriculture for ± 6 months.

2.2. Research Procedure

Fusarium isolate was obtained from chilis raised by farmers. These plants displayed wilting symptoms. Fusarium isolates were propagated with standard method using potato dextrose agar which had been sterilized using an All American Electric Autoclave Sterilizer 50X at 121ºC with a pressure of 1 atm. One capsule of 250 mg Chloramphenicol per liter was applied to PDA to prevent bacteria growth. The media was incubated for 3 days to identify potential contamination. Fusarium was grown on sterile media using a 1-cm-diameter cork borer and incubated for 10 days.

Retrieved from the forest, soil as planting medium was sifted and sterilized through steam sterilization for ± 10 hours using iron barrel. The polybag was 30 cm in diameter and 40 cm in height. Sterile soil was put in 5 kg of polybags and combined with 100 grams of chicken manure as fertilizer.

Fusarium grown in a petri dish was dissolved using sterile aquadesh to obtain a spore density of 7.6×10^5 conidia per ml. One week before the experiment, 20 ml of Fusarium suspension per polybag was applied to the growing medium (soil + chicken manure).

Planting began by creating a hole 2 cm deep and then placing 2 asparagus bean seeds per hole in the polybag. The seeds were covered with soil and then watered. The distance between polybags was 40 cm x 40 cm. The seeds were maintained to ensure decent health and productivity. Watering was done twice per day using 250 ml of water per polybag in each watering unless it rained. Weeding was done manually around asparagus beans and polybags. Thinning of plants with low growth was done 2 weeks after planting, resulting in only one plant in each polybag. Crutches were installed at the same age. At that age, the plants started to need a crutch as a medium for propagation. The crutch was made of wooden sticks with a diameter of 0.5 – 1 cm and a height of 1.5 – 2 m, and it was placed 5 cm from the plants, so the crutch would not reach the roots during installation.

The botanical pesticide was produced using fresh leaves one day before application. Producing botanical pesticides with a concentration of 5% required 75 grams of washed leaves, while 150 grams of leaves were required for a 10% concentration. Once cleaned, the leaves were dried and then chopped. The leaves were soaked using 1,500 ml of water and let dry for 24 hours before being filtered. In the 1st, 3rd, and 5th week after planting, 100 ml of botanical pesticides were applied in each polybag, focusing on the soil and plant parts. Afterward, the treatments were administered, involving sterile distilled water (control), 5% neem leaf extract, 10% neem leaf extract, 5% betel leaf extract, and 10% betel leaf extract. This study used a one-factor randomized block design. Each treatment was repeated 5 times and each treatment consisted of 3 observation polybags so the total experimental units are 75.

2.3. Observation Parameters

The Number of Affected Branches

The number of affected branches on each plant was observed from the 2nd to the 6th week after planting.

The Number of Pods

The number of pods was calculated by adding up all the pods from the first to the last harvest in each plant.

Pod Weight

The pod weight from each sample plant was calculated by adding up the pod weight from the entire harvests using an analytical balance.

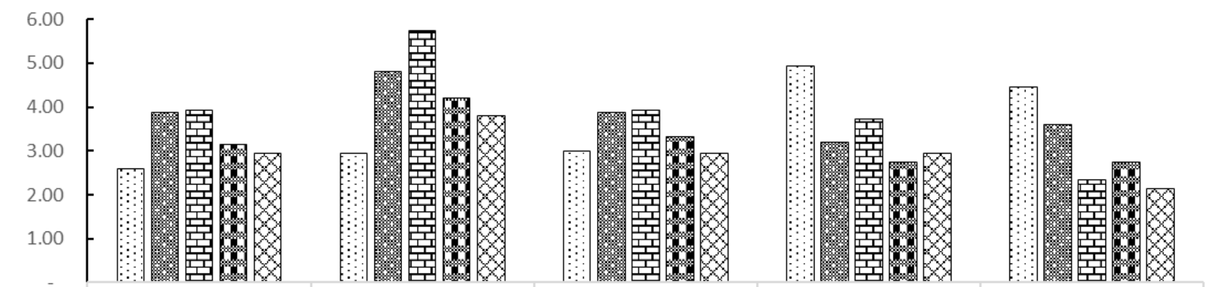
3. Results and Discussion

A genus of *filamentous* fungus, Fusarium consists of multiple species found in a wide range of habitats globally (Egbuta *et al*., 2016). This genus belongs to the class of *Sordariomycetes* and the family of *Nectriaceae* (Vu *et al*., 2019). It has a distinctive structure called sporodochia, which is an aggregate of microscopic structures called conidiophores. The shapes of sporodochia can be plates, stars, or like cotton. *Conidiophores* are structures consisting of fine, slender threads that constitute a clustered network in *sporodochia*. At the ends of the *conidiophores*, there are various asexual reproductive structures called *conidia*. *Fusarium conidia* generally appear elliptical and brightly colored, such as yellow, orange, or brown. *Fusarium* also has fine, insulating threads called hyphae, which are pivotal for collecting nutrients and connecting various parts of the fungus. The morphological structure of Fusarium plays an important role in the process of infecting plants. Kakouridis *et al*. (2022) mentioned that external hyphae can produce spores that can move through the air or soil, and when these hyphae enter the plant, they can damage plant tissue. Fusarium has a special structure called an appressorium. The appressorium is a ring-shaped structure that allows Fusarium to attach plant surfaces and penetrate plant tissue (Doehlemann & Hemetsberger, 2013). In addition, Fusarium can produce mycotoxins which can damage plant tissue and disrupt plant growth (Perincherry *et* al., 2019). Once Fusarium manages to enter plant tissue, it can spread and cause disease symptoms. *Fusarium* infects many plant parts, including roots, stems, leaves, and fruits with such effects as wilting, root rot, stem base rot, leaf spot, and fruit abscission.

Before planting, Fusarium pathogens had been infected in compliance with its characteristics as a soil-borne pathogen. Di *et al*. (2016) stated that Fusarium is a water- and soil-borne pathogen.

In addition, Cahyaningrum *et al.* (2017) explained *that Fusarium oxysporum* f.sp zingiberi is a fungal pathogen that can survive in the soil for a long time. Ploetz (2006) further claimed that the fungus consists of several races and strains with different levels of virulence, and it can survive in soil without a main host for up to 40 years. Likewise, Zhao *et al.* (2020) mentioned that Fusarium can survive in the soil in the form of chlamydospores, and Henry *et al.* (2020) argued that Fusarium can also survive as a saprophyte in plant residues or other organic materials. During its resting phase, Fusarium will be active in the presence of a suitable host.

The Average Number of Wilted Branches shows they are not significantly different (Figure 1). M3 documented a higher average number of wilted branches compared to M2 in all treatments. Meanwhile, the effect of botanical pesticides was marked in M4, which was 3 weeks after the first extract application. In (P1) where the growing medium was infected with Fusarium but uncontrolled, the average number of wilted stems increased until the 6th week. In the 2nd week, the average number of wilted stems was 2.60, and it increased to 4.47 in the 6th week, implying that the extract takes longer time to work than synthetic chemical pesticides. To this end, Tampubolon *et al.* (2018) stated that one of the disadvantages of botanical pesticides is their slow effects. Grdiša and Gršić (2013) added that synthetic chemical pesticides have fast action. Suanda and Sumarya (2021) also reported that botanical pesticides prevented the chili from dying due to the Fusarium pathogen only after the third week of observation.



	M2	M3	M4	M5	M6
P0	2.60	2.93	3.00	4.93	4.47
P1	3.87	4.80	3.87	3.20	3.60
P2	3.93	5.73	3.93	3.73	2.33
P3	3.13	4.20	3.33	2.73	2.73
P4	2.93	3.80	2.93	2.93	2.13

Figure 1. The Average Number of Wilted Branches

Notwithstanding, this slow effect does not reduce the benefits of botanical pesticides in production parameter. The commercial value of the asparagus bean resides in the pod. Fewer wilted branches due to the application of botanical pesticides afford the potential of a higher production rate, as observed from the number and weight of the pods. Analysis of covariance shows that the application of botanical pesticides as a treatment does not give significant effect on the average number of wilted branches. Even so, the average number of wilted branches decreased due to botanical pesticides treatment. As shown in Table 1, P4 has 19 more pods and a higher total weight of 50 grams than P0. Compared to P0 in Table 1, the difference in the number and the total weight of pods following the application of 10% botanical pesticide (P2=12 pods; P2=51 grams; P4=19 pods; and P4=52 grams) is greater than that in the application of 5% botanical pesticide (P1 = 7 pods, P1 = 44 grams, P3 = 2 pods, and P3 = 17 grams). This is because a 5% concentration leads to lower control of fusarium with a density of 7.6 x 10^5 conidia per milliliter than a 10% concentration. By implication, a higher concentration of botanical pesticides leads to a greater possibility of inhibiting fungus growth and reproduction. High fungicide concentration can inhibit fungus growth and reduce spore density. However, there remains a certain threshold of concentration for effective progress to take place.

Table 1. The Observation of Production Parameters

Treatment	The Number of Pods	The Weight of Pods (Grams)
P0	58	3220
P1	65	3264
P2	70	3271
P3	60	3237
P4	77	3272

Neem extract involves several mechanisms to fight the fungus. It contains such active compounds as azadirachtin, nimbin, and salannin which have microbial growth inhibitory properties (Dewi *et al.*, 2017). These compounds interfere with the fungus life cycle, including spore development, mycelium formation, and the production of enzymes driving fungus survival and reproduction. Compounds in neem extract can also damage fungus cell membranes, causing the leakage of important substances in fungal cells, disrupting osmotic balance, and nullifying fungal cells (Ali *et al.*, 2019). Moreover, several compounds in neem extract can inhibit enzyme activities important to the physiological functions and growth of fungus (Gholamnezhad, 2019).

Betel extract contains active compounds such as eugenol, chavibetol, and hydroxycavibetol (Naja *et al.*, 2022), each of which has growth-inhibitory properties. In addition to interfering with fungus enzyme activity and metabolic processes and damaging cell membranes, botanical pesticides in betel extract inhibit sporulation (Suriani *et al.*, 2021). Spores are an important reproductive form of fungi in the spread of disease (Odebode *et al.*, 2020). Betel extract inhibits the formation and release of fungal spores, reducing the fungus's ability to spread and infect further. Furthermore, the active compounds naturally trigger the production of plant defense compounds against fungal infections, such as phytoalexins, protease enzymes, and other antimicrobial compounds (Kishore & Pande, 2005).

4. Conclusion

Based on data comparisons, the application of neem and betel leaf extracts at 5% and 10% concentrations can reduce the average number of wilted branches due to Fusarium wilt. Reducing the number of wilted branches will increase the total number and the total weight of pods.

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