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Original article

Control of *Fusarium verticillioides* on corn with a combination of *Bacillus subtilis* TM3 formulation and botanical pesticides

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ABSTRACT

The aim of this study was to evaluate the effectiveness of the combination of *Bacillus subtilis* TM3 formulation with botanical pesticides in suppressing *Fusarium verticilloides* infection in corn. The research was carried out at the Plant Pathology Laboratory and the Experimental Farm of Indonesian Cereals Research Institute (ICERI) from February to November 2019. The research consisted of two stages, namely an in vitro test of antagonists of botanical pesticides against *F. verticilloides* using 5 types of plant extracts namely betel leaf extract, turmeric, galangal, cosmos, and clove leaf. The second stage was to test the effectiveness of the combination of the formulation of *B. subtilis* TM3 with the best 3 types of plant extracts in vitro testing in suppressing *F. verticilloides* infection in plants. The results of the in vitro study showed that the plant extracts of betel leaf, clove leaf and galangal had the best inhibitory ability on the mycelia growth of *F. verticilloides*. Meanwhile, the field test found that the application of the *B. subtilis* TM3 formulation, either alone or in combination with plant extracts, was able to suppress *F. verticilloides* infection. The combination of *B. subtilis* TM3 formulation with betel leaf extract showed the best inhibition of 20% against stem rot disease and 13.33% against corn cob rot. This treatment did not affect production quantitatively, but was able to suppress the decline in seed quality due to *F. verticilloides* infection. Seeds grown by the Plastic Rolled Paper Test (PRPT) method were not only infected with *F. verticilloides*, but also infected with other seed-borne pathogens, such as *Aspergillus niger* and *A. falvus*. The presence of these two pathogens did not inhibit the growth of *F. verticilloides* in kernels.

1. Introduction

The fungus *Fusarium verticillioides* is a pathogen that infects corn plants and causes a decrease in production in the world (Li et al., 2019). This pathogen infection reported from several countries that develop corn can reduce production in the range of 10–50%. (Venturini et al., 2011); Li et al., 2010). In Indonesia, the decrease in production due to this pathogen is not widely reported, but the decline in the quality of corn kernels from the accumulation of the pathogen results in low selling value (Pakki, 2016). Infection on the ears is a major concern because the toxin produced by this pathogen is in the form of fumonisin which can accumulate in kernels and is harmful to human and livestock health (Zhang et al., 2013; Rocha et al., 2016). Accumulation of fumonisin in the body can inhibit sphingolipid metabolism and cell cycle regulation resulting in effects as diverse as oesophagal cancer and the risk of neural tube defects in children. Based on this, it is necessary to control this disease to reduce farmers' losses. The control of stem rot disease is commonly carried out by spraying fungicides on plants. Meanwhile, the public has now felt the negative impacts that arise on human health due to the accumulation of pesticide residues in agricultural products, so that safe and environmentally friendly control techniques are needed, such as the use of biological agents (Suriani and Muis, 2016). The effectiveness of biological agents in controlling pathogens that cause plant disease has been widely reported.

In the last two years, we tested the effectiveness of 8 *Bacillus subtilis* isolates which were explored from several regions in Indonesia and have been formulated in the form of talc as a biological agent in suppressing the attack of important diseases in maize, including *Fusarium* stem rot disease. The results of the test in vivo provided recommendations for 2 isolates of formulations of *B. subtilis* to effectively control stalk rot, then the two formulations were

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tested in the field with several dosages and the results showed that the formulation of *B. subtilis* TM3 with 2.5 kg./ha was effective in controlling Fusarium stalk rot (Suriani et al. 2020).

The effectiveness of antagonistic microbes in cropping is influenced by several factors including climate, physical and chemical properties of plant surfaces and the environment, and the microbial population. If management is not carried out properly, it will reduce the activity of the biological control agent (BCA). For this reason, the use of BCA can be optimized with a combination of using other control methods, such as adjusting soil pH, crop rotation, immersion of fresh organic matter, environmental modification and the use of botanical pesticides Soesanto et al. (2005). Several research results show the effectiveness of a combination of BCA and botanical pesticides in suppressing pathogen including, tapak liman leaf extract, neem, betel, and citronella have the potential to suppress downy mildew in sweet corn (Sekarsari et al., 2013). Soesanto et al. (2005) suggested that ginger rhizome rot in the land can be controlled by the application of the BCA *Trichoderma* sp. (dose of 20 g plant^{-1}) either singly or in combination with clove leaf powder (dose 10 g of plant^{-1} 4 times every 7 days interval), which is given since the seedlings are planted, and sprinkled around the crops.

Based on the foregoing, this research was conducted to obtain a combination of treatment of the formulation of *B. subtilis* TM3 with botanical pesticides that have the potential to control *F. verticillioides* infection in corn.

2. Material and method

The research was conducted at the Plant Pathology Laboratory and Maros Experimental Farm of Indonesian Cereals Research Institute (ICERI) from February 2019 to November 2019. The activity began with the isolation of the fungus *F. verticillioides* from the stalks of corn plants infected with stalk rot by growing them on DifcoTM Potato Dextrose Agar (PDA), then incubated for 2 days. The fungal mycelium that grew was then re-isolated on new PDA medium and incubated for 14 days. Furthermore, macroscopic identification was carried out including the shape and color of the colony, microscopically by looking at the shape of the conidia of the fungus and then matching it with a reference. Isolates identified as *F. verticilloides* were propagated in Petri dishes as inoculants. The *B. subtilis* TM3 formulation used was the result of development at the Plant Pathology Laboratory of ICERI. The botanical pesticides used were obtained from the Indonesian Vegetable Research Institute, Lembang and consisted of clove leaf extract, galangal extract, cosmos extract, and turmeric rhizome extract.

2.1. Antagonist test of botanical pesticides against F. verticillioides in vitro

The treatment was arranged in a completely randomized design, consist of six treatments (five extracts of botanical pesticides and sterile distilled water as control) and 4 replicates. Each botanical pesticide was diluted with a concentration of 2%, then each was poured 1 ml into a Petri dish contains 10 ml of PDA media. Shake the petri dish to make it homogeneous, then let stand for 10 min so that the suspension was absorbed by the media. Pieces of *F. verticillioides* (1 cm diameter) were placed in the center of the petridish, then incubated for 7 days. In the control treatment, PDA medium was given 1 ml of sterile distilled water and then *F. verticillioides* was grown in the center of the petri dish. The level of inhibition of botanical pesticides on the growth of *F. verticillioides* was were observed by measuring the development of *F. verticillioides* mycelia in each treatment then converting the following formula:

$$THR = \frac{dk - dp}{dk} \times 100\%$$

where THR is the relative inhibitory level (%), dk (fungus diameter on control) and dp (fungus diameter on treatment). Test results were analyzed using a variety of fingerprints. If there is a difference in treatment data with analytic control continued with Duncan Test at 5%.

2.2. The effectiveness test of the combination formulation of B. subtilis and botanical pesticides in controlling Fusarium verticillioides in crops

Preparation made before the implementation of the test in the field was the propagation of *F. verticillioides* using the toothpick contamination method (Nordby et al., 2007). Testing the ability of the combination of *B. subtilis* formulation and botanical pesticides to control *F. verticillioides* in corn was carried out at the Maros Experimental Farm of ICERI. The treatments were arranged in a randomized block design consisting 8 treatments with 3 replicates. The eight treatments were single application formulation *B. subtilis* TM3 (P1), formulation *B. subtilis* TM3 + betel leaf extract (P2), formulation *B. subtilis* TM3 + clove leaf extract (P3), formulation *B. subtilis* TM3 + galangal extract (P4), control with active ingredient fungicide difenoconazole (P5), control with a fungicide with active ingredient mancozeb (P6), positive control (sterile distilled water with inoculation of *F. verticillioides*) (P7) and negative control (sterile distilled water without inoculation of *F. verticillioides*) (P8).

The first step was planting NEI 9008 line on a treatment plot measuring 3.75 × 5 m, one plant per hole with a planting space of 75 cm × 20 cm. Furthermore, maintenance was carried out including fertilization, weed control, and plant watering. Fertilization was carried out twice, the first fertilization was applied 10 days after planting (DAP) using urea and NPK fertilizers with doses of 150 kg/ha and 200 kg/ha, respectively. While the second fertilization was applied at 30 DAP using Urea fertilizer at a dose of 150 kg/ha. Inoculation of *F. verticilliodies* was done artificially at 45 DAP by making a hole of 0.25 cm in the second stem segment and then inserting a toothpick that had been covered with *F. verticilliodies*. The application of the *B. subtilis* formulation was carried out in 2 ways (seed treatment and spraying). Spray application was carried out 7 days after inoculation of *F. verticilliodies*, at a dose of 2.5 kg/ha. Meanwhile, the application of botanical pesticides was carried out 14 days after inoculation of *F. verticilliodies* at a dose of 4 l/ha. Each pesticide was sprayed on the surface of the plant evenly.

The variables observed included the severity of Fusarium stalk rot, the severity of Fusarium ear rot disease, yield, and the percentage of seeds infected with *F. verticillioides*.

2.2.1. Disease incidence of Fusarium stalk rot

Observation on the severity of Fusarium stalk rot was done at 75 and 90 DAP by determining the damage score adopted from (Ahamad et al., 2015) as follows: score 1 = healthy or slight color change in the first stem internode; score 2 = up to 50% of the first segment changes color; score 3 = 51–75% of the first segment changes color; score 4 = 76–100% of the first segment changes color; score 5 = 50% change in color of the first segment adjacent; score 6 => 50% change in color of adjacent internodes; score 7 = change in color of three internodes; score 8 = change in color of four internodes; and score 9 = change in color of five or more internodes and premature death of the plant. Crop damage scores are converted to the following formula:

$$DS = \frac{\sum(n_i \times V_i)}{Z \times N} \times 100\%$$

where: DS = Disease severity (%).

n_i = infected plant to-i;
v_i = score by category of infection to-i;
N = Number of plant observed;
Z = The highest score of infection category (score 9).

2.2.2. The severity of Fusarium ear rot

This variable was observed at harvest by taking 20 samples of ear per treatment unit. Ear damage scores converted to disease severity. The scores for ear damage due to *F. verticillioides* infection were: score 1 = 1–3% of corn kernels infected with the disease; score 2 = 4–10% of corn kernels infected with disease; score 3 = 11–25% of corn kernels infected with disease; score 4 = 26–50% of corn kernels infected with disease; score 5 => 75% of corn kernels infected with disease; score 6 = 4–10% of corn kernels infected with disease (Reid et al., 1993).

2.2.3. Yield

Observations were made on several aspects of the harvest including the weight of wet peeled ears, shelling percentage, and moisture content. The results of the observations were converted to yield/ha using the following formula:

$$yield(t/ha) = \frac{10.000}{HA} \times \frac{100 - MC}{100 - 15} \times PW \times SP$$

where: HA = Harvested area (m^2).
MC = Moisture content at harvest (%).
PW = Wet peeling weight (kg).
SP = Shelling percentage (%).

2.2.4. Percentage of seeds infected with F. verticillioides

This step was carried out at the Plant Pathology Laboratory of ICERI. A total of 100 kernels from each research unit were grown using the Plastic Rolled Paper Test (PRPT) method and stored for 7 days. The percentage of seeds infected is calculated according to the following formula:

$$I = \frac{A}{B} \times 100\%$$

where: I = Percentage of *F. verticilloides* infected kernels.
A = Number of *F. verticilloides* infected kernels
B = Number of observed kernels.

The data for each observation variable was analyzed separately using the computer program STAR Ver. 2.0.1 for Windows. Variance and the mean of the treatments are differentiated with the Duncan's Multiple Range Test (P = 0.05).

3. Result

3.1. Antagonist test of botanical pesticides against F. verticillioides in vitro

The test results of the inhibition of five botanical pesticides against the growth of *F. verticillioides* mycelia showed that betel leaf extract had the highest inhibition of 71.12%, which was significantly different from the inhibition ability of 3 other extracts (cosmos, galangal and turmeric) (Table 1). The growth of *F. verticillioides* mycelia for 9 days of storage in the betel leaf extract treatment was very slow with a mycelial growth diameter of ±2 cm, while the mycelia growth without the use of botanical pesticides (control) filled the Petri dishes with a diameter of 9 cm (Fig. 1).

Table 1
Inhibition of 5 botanical pesticides on the growth of *F. verticillioides* mycelia in vitro.

No	Treatment	Percentage of mycelial inhibition of *F. verticillioides* (%)	
1	Clove leaf extract VS *F. verticillioides*	67.32	ab
2	Cosmos extract VS *F. verticillioides*	61.49	b
3	Tumeric extract VS *F. verticillioides*	56.14	c
4	Galangal extract VS *F. verticillioides*	63.36	b
5	Betel extract VS *F. verticillioides*	71.12	a
6	Control (*F. verticillioides)*	0.00	d

Note: The number in a column followed by the same letter is not significantly different at the 5% level of the DMRT.

3.2. The effectiveness test of the combination formulation of B. subtilis and botanical pesticides in controlling Fusarium verticillioides in crops

The results of observations on the severity of fusarium stalk rot disease showed that the combined application of the formulation *B. subtilis* TM3 and botanical extracts suppressed disease progression higher than the single application of the formulation *B. subtilis* TM3, but it did not show a significant difference. Plants that were applied a combination of the formulation *B. subtilis* TM3 and betel leaf extract showed the lowest disease severity, namely 25.19%, not significantly different from the application of the two types of synthetic pesticides which showed the incidence of Fusarium stalk rot disease with 24.44% and 21.48% respectively at 90 DAP (Table 2). Plants in negative control were also found to be infected with pathogens on both stems and cobs, but the severity of stem rot and cob rot in negative controls was very low (Tables 2 and 3). The emergence of disease in plants that are not inoculated can be caused by surface water flow that can spread this pathogen. It is known that *F. verticilloides* is a soil-borne pathogen.

F. verticillioides infection on corn stalks then spreads and infects the ears, causing damage and rot in the kernels. The application of BCA in this study had lower effectiveness in suppressing ear rot infection compared to the use of synthetic pesticides, either difekanozole or mancozeb (Table 3). However, the combined application of the *B. subtilis* formulation and betel leaf extract consistently reduced the incidence of *F. verticillioides* both on the stalks and ears.

The test for the accumulation of *F. verticillioides* on the harvested kernels was observed in the laboratory by growing the seeds on test paper rolled in plastic (TPRP) media. The results showed that the kernels were infected with *F. verticillioides* evenly and did not show any significant differences between treatments. The lowest infected kernels were in a single application of the *B. subtilis* TM3 formulation, which was the same as the infection in the mancozeb treatment of 8.33% (Fig. 2). Infected kernels were found to have purplish-white mycelia and conidia which were observed microscopically in oval shape (Fig. 3).

Besides being infected with *F. verticillioides*, seeds grown on TPRP media were also found *Aspergillus flavus* and *A. niger* with different infection characteristics in the kernels (Fig. 4). Both fungi are transmitted through seeds and produce harmful toxins such as *F. verticilloides*.

The yield in this experiment showed that there was no significant different among the treatments (Fig. 5). However, there was a decrease in production due to corn stem rot disease. This can be seen in plants that were not inoculated with *F. verticilloides* (negative control) showing higher yield than that in plants inoculated with *F. verticilloides*, both in the treatment with pesticides and in plants without the application of pesticides (positive control). However, the production of pesticide application was lower than the production of positive control.

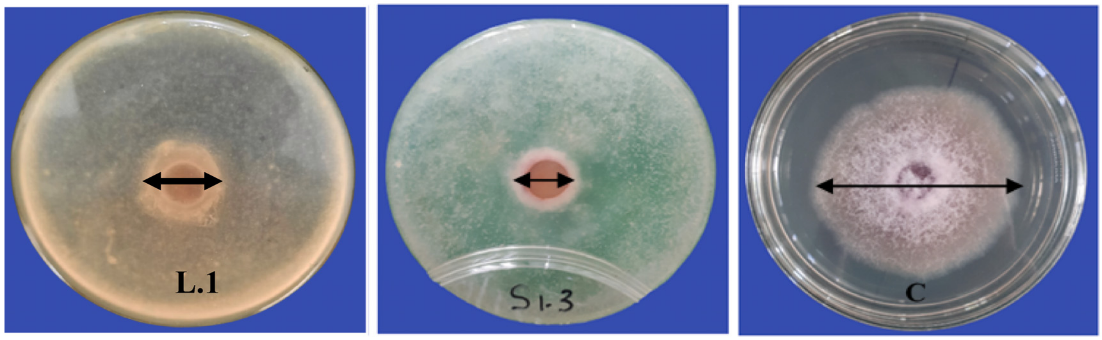

Fig. 1. Inhibition test of botanical pesticides against F. verticillioides (L.1: galangal extract vs F. verticillioides; S1: betel leaf extract vs F. verticillioides; C: control).

Table 2
Effect of application of a combination of the formulation of B. subtilis TM3 and botanical pesticides on the attack of Fusarium stalk rot in Maros experimental farm, 2019.

Treatments	Disease severity at (%)			
	75 DAP		90 DAP	
B. subtilis TM3 formulation	12.59	bc	27.41	bcd
Combination of *B. subtilis* TM3 formulation and betel leaf exctract	14.81	ab	25.19	cd
Combination of *B. subtilis* TM3 formulation and clove leaf extract	14.07	abc	27.41	bcd
Combination of *B. subtilis* TM3 formulation and galangal extract	14.07	abc	25.93	cd
Difenokonazol fungicide	11.11	bc	24.44	cd
Mankozeb fungicide	12.59	bc	21.48	d
Inoculation of *F. verticillioides* without any pesticide (control positive)	19.26	a	45.19	a
Without inoculation of *F. verticillioides* (control negative)	1.48	d	2.96	e
DMRT value (5%)	6.54		7.67	

Note: The number in a column followed by the same letter is not significantly different at the 5% level of the DMRT.

Table 3
Effect of application of a combination of the formulation of *B. subtilis* TM3 and botanical pesticides on the attack of Fusarium ear rot in Maros experimental farm, 2019.

Treatments	Ear rot disease severity (%)
B. subtilis TM3 formulation	6.67
Combination of *B. subtilis* TM3 formulation and betel leaf exctract	6.19
Combination of *B. subtilis* TM3 formulation and clove leaf extract	6.98
Combination of *B. subtilis* TM3 formulation and galangal extract	9.05
Difenokonazol fungicide	4.29
Mancozeb fungicide	4.64
Inoculation of *F. verticillioides* without any pesticide	19.52
Without inoculation of *F. verticillioides*	7.14

Note: The number in a column followed by the same letter is not significantly different at the 5% level of the DMRT.

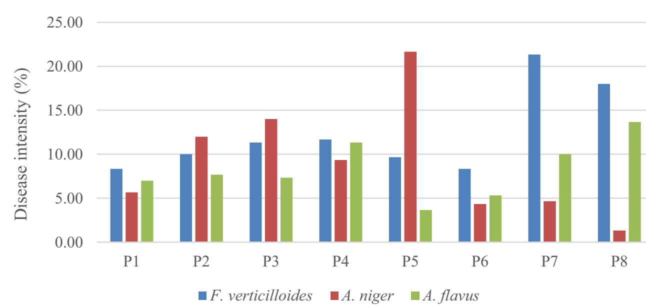

Fig. 2. Infection rates of *F. verticillioides* and 2 other seed-borne pathogens against corn kernels in a combined application of the formulation *B. subtilis* TM3 and botanical pesticides. (P1: Formulation *B. subtilis* TM3; P2: Combination of Formulation *B. subtilis* TM3 and betel leaf extract; P3: Combination of Formulation of *B. subtilis* TM3 and clove leaf extract; P4: Combination of Formulation of *B. subtilis* TM3 and galangal extract; P5: difenoconazole fungicide; P6: Mankozeb fungicide; P7: *F. verticillioides* inoculation without biopesticide; P8: Without *F. verticillioides* inoculation.

4. Discussion

The mechanism of betel leaf extract in inhibiting the growth of *F. verticillioides* is not widely known. However, previous researchers reported that the mechanism of antifungal activity of betel leaf extract of Beleng cultivar against the fungus *F. oxysporum* f. sp. *vanillae* occurs through inhibition of spore germination, spore formation and colony growth (Subrata and Rai, 2019). The ethanol content in black betel leaf extract has the highest inhibitory activity compared to other compounds contained in betel leaves such as n-hexane and ethyl acetate extract (Junairiah et al., 2017).

Other botanical pesticides that have high inhibition of the growth of *F. verticillioides* mycelia besides betel leaf extract were clove and cosmos leaf extracts with inhibition percentage of 67.32% and 63.36%, respectively. The results of this test underlie the use of 3 types of botanical extracts combined with the formulation of *B. subtilis* in controlling *F. verticillioides* infection in corn.

The biological control carried out in the field shows the consistent effectiveness of the combined formulation of *B. subtilis* and betel leaf extract in suppressing the infection of *F. verticilloides* both on the stalk and on the ear. The effectiveness of betel leaf extract in suppressing *F. verticillioides* is might be due to the high content of phenolic compounds which are antifungal in betel leaf (Susanti et al., 2017). Phenolic compounds can break the cross-linkage (cross-linkage) of peptidoglycan and penetrate the fungal cell wall (Ingram, 1981).

The inhibition ability of the combination *B. subtilis* TM3 formulation and botanical pesticides against *F. verticillioides* in the plant was not significantly different from the single application of *B. sub-*

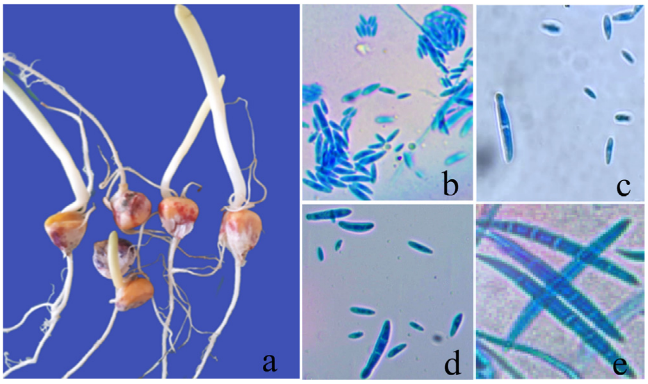

Fig. 3. (a) Symptoms of *F. verticillioides* infection in corn kernels; (b) Microconide detached from the apical end of the monophialide; (c-d) aerial conidia with 1–2 septa; e. Macroconidia *F. verticillioides* with 3–5 septa.

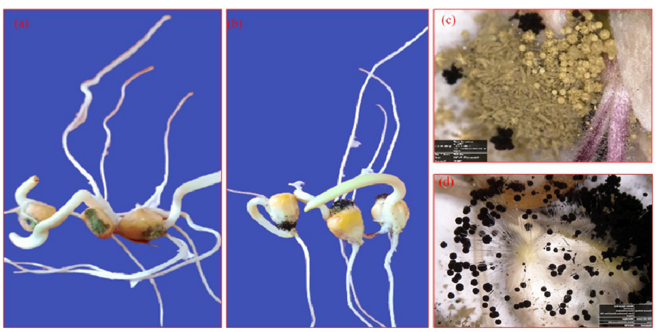

Fig. 4. Seeds infected with *A. flavus* (a) and *A. niger* (b), *A. flavus* (c) and *A. niger* (d) conidiophores growing on the surface of the seeds.

tilis TM3 formulation. Unlike the case with the test results on the mycelial growth inhibition of *F. verticillioides* using betel leaf extract in vitro, the inhibition ability was 71.12%. The decrease in the anti-fungal ability of botanical pesticides applied to corn plant is due to the content of betel leaf extract in the form of volatile essential minerals at room conditions or higher temperatures (Hartati, 2012).

The combination application of the *B. subtilis* formulation with plant extracts in this study did not have an effect on increasing yield, although in general it was able to suppress the severity of stem rot disease. Even the yield with the application of the disease control agent was lower than plants with higher disease severity (positive control). This can happen because plants that are attacked by stem rot at 90 DAP generally have entered the physiological maturation phase of the cob so that they can still be harvested but the quality of the seeds produced decreases. Positive control plants that experienced the highest stem rot disease severity also showed high levels of *F. verticilloides* infection on the cobs and seeds (Table 3 and Fig. 2). Infections in the cobs and seeds from positive control plants were highest compared to other treatments, which were 19.52% and 21.33%, respectively, while the *B. subtilis* formulation and plant extracts showed the lowest infections, cob infected with 6.19% on seeds by 10%. This of course will reduce the quality of the seeds both to be used as animal feed and plant seeds. In addition to the accumulation of toxins in the form of fumonisin which are harmful to livestock health, seeds infected with *F. verticilloides* if planted will become a source of inoculum and cause systematic infection, a cycle of infection from seed to stem and so on will infect newly formed seeds (Gai et al., 2018).

Fusarium verticilloides was found in seeds obtained from the infected plants. White to pinkish fungal hyphae were colonized the seeds grown in the laboratory as shown in Fig. 3 with oval-shaped conidia with septa. This is in accordance with the identification made by (Pelizza et al., 2011), the microscopic characteristics of *F. verticillioides* were found to be oval, without septae and 6–10 $\times$ 1.5–2.5 microconidia, while macroconidia had 3–5 septa. Conidia produced by *F. verticillioides* infects the kernels, causing a pink to brown discolouration of the kernel surface (Duncan and Howard, 2010). In addition to *F. verticilloides* which infects seeds, this study also found *A. flavus* and *A. niger* infections. The growth of *A. niger* was characterized by the presence of black hyphae and conidiaphores surrounding the seeds, while *A. flavus* had green mycelia growing on the seed surface (Sreenu et al., 2019). *F. verticilloides, A. niger* and *A. flavus* have similarities in their growth environment, but *F. verticilloides* can inhibit the growth of both fungi, especially at $> 0 \pm 96$ activity water (Marin et al., 1998; Thompson and Raizada, 2018).

Kernels infected with *F. verticilloides, A. niger and A. flavus* similar to this study should not be used as animal feed or other processed food materials because the mycotoxins produced by these microbes are harmful (García-Díaz et al., 2020; Puspitasari et al.,

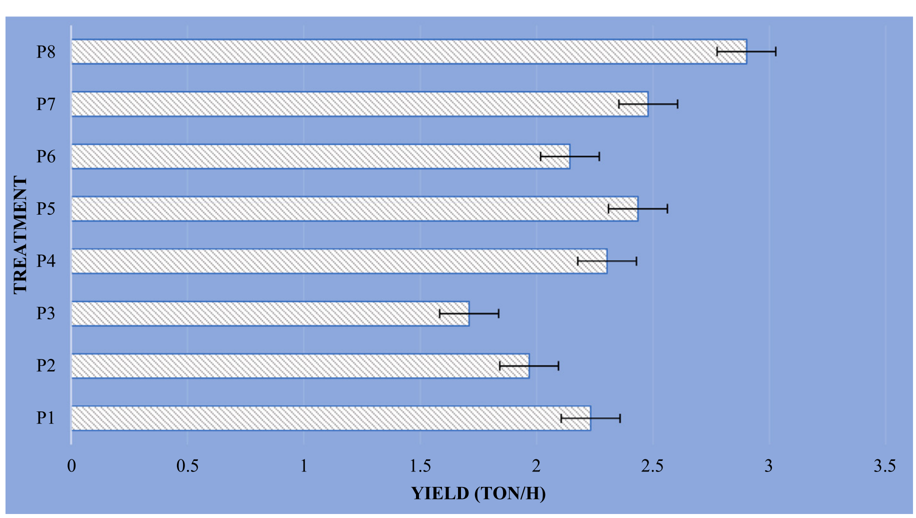

Fig. 5. Average yields on the treatment combination of *B. subtilis* TM3 formulation and botanical pesticides. Maros experimental farm, 2019. (P1: *B. subtilis* TM3 formulation; P2: Combination of *B. subtilis* TM3 formulation and betel leaf extract; P3: Combination of *B. subtilis* TM3 formulation and clove leaf extract; P4: Combination of *B. subtilis* TM3 formulation and galangal extract; P5: Diphenoconazole; fungicide P6: Mankozeb fungicide; P7: Inoculation of *F. verticillioides* without biopesticide; P8: Without inoculation of *F. verticillioides*.

2015). Mycotoxin contamination is one of the major post-harvest problems of agricultural products in Indonesia (Miskiyah et al., 2010). The content limit of some mycotoxins has been regulated in agricultural products in many countries including aflatoxins produced by *Aspergillus* spp. and fumonisin from the Fusarium group (Bagus et al., 2017). Therefore, the control of *F. verticilloides* is necessary to ensure the quality of agricultural products produced.

5. Conclusion

1. In vitro test of the inhibitory power of plant extracts on the mycelia growth of *F. verticilloides* found 3 plant extracts that had the best inhibitory power, namely betel leaf extract, clove leaf extract and galangal extract.
2. The application of the *B. subtilis* TM3 formulation, either alone or in combination with plant extracts as a whole, was able to suppress *F. verticilloides* infection in corn plants. The combination of *B. subtilis* formulation with betel leaf extract showed the best inhibition of 20% against stem rot and 13.33% against cob rot. This treatment did not affect the yield quantitatively, but was able to suppress the decrease in the quality of the seeds produced.
3. Seeds grown using the PRPT method, apart from being infected with *F. verticilloides*, corn seeds were also infected with other seed-borne pathogens, namely *A. niger* and *A. falvus*. The presence of these two pathogens did not inhibit the growth of *F. verticilloides* in seeds.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Ahamad, S., Lal, B., Kher, D., 2015. Screening of maize germplasms against stalk rot diseases in the intermediate zone of Jammu region. Int. J. Innov. Sci. Eng. Technol. 2, 1024–1032.

Bagus, I.G.N., Widaningsih, D.W.I., Sudarma, D.A.N.I.M., 2017. Keragaman Jamur yang Mengkontaminasi Beras dan Jagung di Pasar Tradisional Denpasar. Agrotrop 7, 89–98.

Duncan, K.E., Howard, R.J., 2010. Biology of maize kernel infection by Fusarium verticillioides. Mol. Plant-Microbe Interact. 23, 6–16. https://doi.org/10.1094/MPMI-23-1-0006.

Gai, X., Dong, H., Wang, S., Liu, B., Zhang, Z., Li, X., Gao, Z., 2018. Infection cycle of maize stalk rot and ear rot caused by Fusarium verticillioides. PLoS One 13, 1–11. https://doi.org/10.1371/journal.pone.0201588.

García-Díaz, M., Gil-Serna, J., Vázquez, C., Botia, M.N., Patiño, B., 2020. A comprehensive study on the occurrence of mycotoxins and their producing fungi during the Maize production cycle in Spain. Microorganisms 8. https://doi.org/10.3390/microorganisms8010141.

Hartati, S.R.I.Y., 2012. Prospek Pengembangan Minyak Atsiri sebagai Pestisida Nabati. Perspektif 11, 45–58.

Ingram, L.O., 1981. Mechanism of lysis of Escherichia coli by ethanol and other chaotropic agents. J. Bacteriol. 146, 331–336. https://doi.org/10.1128/jb.146.1.331-336.1981.

unairiah, Matuzahroh, N., Zuraidassanaaz, N. istighfari, Sulistryorini, L., 2017. Bioscience Research. Biosci. Res. 14, 750–755.

Li, L., Qu, Q., Cao, Z., Guo, Z., Jia, H., Liu, N., Wang, Y., Dong, J., 2019. The relationship analysis on corn stalk rot and ear rot according to fusarium species and fumonisin contamination in kernels. Toxins (Basel). 11. https://doi.org/10.3390/toxins11060320.

Li, W.J., He, P., Jin, J.Y., 2010. Effect of potassium on ultrastructure of maize stalk pith and young root and their relation to stalk rot resistance. Agric. Sci. China 9, 1467–1474. https://doi.org/10.1016/S1671-2927(09)60239-X.

Marín, S., Sanchis, V., Ramos, A.J., Vinas, I., Magan, N., 1998. Environmental factors, in vitro interactions, and niche overlap between Fusarium moniliforme, F. proliferatum, and F. graminearum, Aspergillus and Penicillium species from maize grain. Mycol. Res. 102, 831–837. https://doi.org/10.1017/S0953756297005777.

Miskiyah, Winarti, C., Broto, W., 2010. Kontaminasi Mikotoksin pada Buah Segar dan Produk Olahannya serta Penanggulangannya. Kontam. Mikotoksin pada Buah Segar dan Prod. Olahannya serta Penanggulangannya 29, 79–85. https://doi.org/10.21082/jp3.v29n3.2010.p79-85.

Nordby, J.N., Pataky, J.K., White, D.G., 2007. Development of Gibberella ear rot on processing sweet corn hybrids over an extended period of harvest. Plant Dis. 91, 171–175. https://doi.org/10.1094/PDIS-91-2-0171.

Pakki, S., 2016. Mycotoxin contamination, bioecology of Fusarium verticillioides pathogen and its control on maize. / Cemaran mikotoksin, bioekologi patogen Fusarium verticillioides dan upaya pengendaliannya pada jagung. J. Penelit. dan Pengemb. Pertan. 35, 11–16.

Pelizza, S.A., Stenglein, S.A., Cabello, M.N., Dinolfo, M.I., Lange, C.E., 2011. First record of Fusarium verticillioides as an entomopathogenic fungus of grasshoppers. J. Insect Sci. 11. https://doi.org/10.1673/031.011.7001.

Puspitasari, D.P. indah, Widiastuti, A., Wibowo, A., Priyatmojo, A., 2015. Intensity of fungal contamination on cattle-feed maize during storage period. J. Perlindungan Tanam. Indones. 19, 27–32.

Reid, L.M., Spaner, D., Mather, D.E., Bolton, A.T., Hamilton, R.I., 1993. Resistance of maize hybrids and inbreds following silk inoculation with three isolates of Fusarium graminearum. Plant Dis. https://doi.org/10.1094/PD-77-1248.

Rocha, L.O., Barroso, V.M., Andrade, L.J., Pereira, G.H.A., Ferreira-Castro, F.L., Duarte, A.P., Michelotto, M.D., Correa, B., 2016. FUM gene expression profile and fumonisin production by *Fusarium verticillioides* inoculated in Bt and non-Bt maize. Front. Microbiol. 6, 1–10. https://doi.org/10.3389/fmicb.2015.01503.

Sekarsari, R.A., Joko, P., Tri, M., 2013. Pengaruh Beberapa Fungisida Nabati Terhadap Keterjadian Penyakit Bulai Pada Jagung Manis (Zea mays saccharata). J. Agrotek Trop. 1, 98–101.

Soesanto, L., Sudarmono, Prihatiningsih, N., Manan, A., Iriani, E., Pramoni, J., 2005. Potensi Agensia Hayati dan Nabati dalam Mengendalikan Penyakit Busuk Rimpang Jahe 5, 50–57.

Sreenu, B., Girish, A.G., Alice, J., Sujeetha, R.P., 2019. Identification and Detection of Maize Seed Borne Pathogens using Different Seed Testing Methods. Int. J. Curr. Microbiol. Appl. Sci. 8, 1460–1466. https://doi.org/10.20546/ijcmas.2019.810.171

Subrata, I.M., Rai, I.G.A., 2019. Aktivitas Fungisida Ekstrak Daun Sirih (Piper Betle L .) Kultivar Beleng terhadap Jamur Fusarium Oxysporum f . sp . Vanillae Penyebab Penyakit Busuk Batang pada Vanili VIII, 41–50.

Suriani, Djaenuddin, N., Muis, A., 2020. Utilization of antagonistic bacteria Bacillus subtilis to control Fusarium verticilloides on corn. IOP Conf. Ser. Earth Environ. Sci. 484. https://doi.org/10.1088/1755-1315/484/1/012100.

Suriani, Muis, A., 2016. Prospek Bacillus subtilis sebagai Agen Pengendali Hayati Patogen Tular Tanah pada Tanaman Jagung. J. Penelit. dan Pengemb. Pertan. 35, 37. https://doi.org/10.21082/jp3.v35n1.2016.p37-45.

Susanti, N.M.P., Dewi, L.P.M.K., Manurung, H.S., Wirasuta, I.M.A.G., 2017. Identification of phenol compond in green piper betle leaf ethanol extract by the TLC-spectrophotodensitometry. J. Metamorf. 4, 108–113.

Thompson, M.E.H., Raizada, M.N., 2018. Fungal pathogens of Maize gaining free passage along the silk road. Pathogens 7. https://doi.org/10.3390/pathogens7040081.

Venturini, G., Assante, G., Vercesi, A., 2011. Fusarium verticillioides contamination patterns in Northern Italian maize during the growing season. Phytopathol. Mediterr. 50, 110–120. https://doi.org/10.14601/Phytopathol_Mediterr-8680.

Zhang, L., Wang, J., Zhang, C., Wang, Q., 2013. Analysis of potential fumonisin-producing Fusarium species in corn products from three main maize-producing areas in eastern China. J. Sci. Food Agric. 93, 693–701. https://doi.org/10.1002/jsfa.5794.