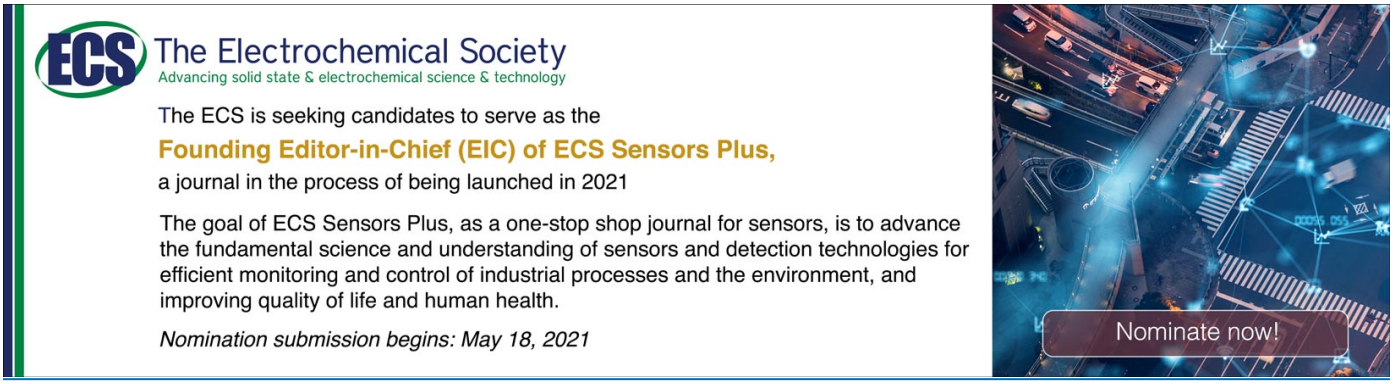
PAPER • **OPEN ACCESS**

The phytopatological compatibility of sunflower (*Helianthus annuus L.*) var. IPB Bm 1 as refugia

To cite this article: A Wafa and Y A Cahyadi 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **759** 012015



The phytopatological compatibility of sunflower (*Helianthus annuus L.*) var. IPB Bm 1 as refugia

A Wafa[1*] and Y A Cahyadi[2]

[1]Plant Protection Department, University of Jember, Jl. Kalimantan No 37, Jember East Java, 68121, Indonesia
[2] Agrotechnology Department, University of Jember, Jl. Kalimantan No 37, Jember East Java, 68121, Indonesia
[*]E-mail : ali.wafa@unej.ac.id

Abstract. Refugia has been used to avoid insect pests in the leguminous plant. However, utilization of refugia never been evaluated at the level of sensitivity to primary plant disease. The research aimed to find out the compatibility of Sunflower Var IPB Bm 1 as refugia due to primary plant pathogen. It affected the stem rot disease on the most crop, caused by the fungal pathogen, *Sclerotium rolfsii*. The Research conducted in the in-vitro level. The sunflower seed tested on the *S. rolfsii* colony by used two different media, planted by 7 mm from the outer colony of fungal. The infection ability, time requirement of infection, sclerotia size, and developing time of sclerotia used as observation subject and compared to the control. Based on the result, compared due to the control treatment, the infection time requirement of the fungal pathogen to infected sunflower are six hours slower than control, have a bigger sclerotia size, more than 47,680 μm^2 bigger and faster-developed sclerotia. The development stage from hyphae to the well-developed sclerotia in Sunflower seeds are 7,1 days faster than the control treatment. The result indicated the *S.rolfsii* could develop well to the dormancy stage on the Sunflower IPB Bm 1 more faster than the control treatment. In line with that, became highly recommend for adding the phytopatologial aspect on the selecting refugia plant in the future. Aimed to reducing the possibility the refugia plant became alternate host and inoculum source of main pathogen.

1. Introduction

Refugia plant is non-crop were planted at the same time and same place with the crop. Purba et al [1] Its provides spatial or temporal shelter for pest natural enemies, and supports biotic interaction components in ecosystems, such as pollinators or pollinating insects. It has been utilized as one of insect pest management technique[2]. Unfortunately, the current utilize refugia still focused on insect pest population without regards that effect to the plant pathogen inoculum and population in the soil and airborne. On another hand, the plant pathogen has had many host plant with diverse plant genera, and each plant genus can be as a primer, secondary and alternate host of it [3].

Sunflower (*Helianthus annuus L*) known as favourite flowering plant, oiling seed [4] and for refugia, it mostly utilized as refugia or border plant on several crop commodities, it can reduce the population of stem borer [5]. On another hand, the sunflower is known as the alternate host of several plant-pathogen [6,7]. Nor, *S. rolfsii* is the significant plant pathogen in seed crop commodities[8,9]. Became from that, this research aimed to find out the compatibility one of the flowering variety of

sunflower, the IPB Bm 1 as refugia on peanut and other *S. rolfsii* host by phytopathological tested. The test conducted with comparing the development of fungal pathogen, *S.rolfsii* on several media and inoculate them around the sunflower seed and their true host.

2. Material and Methods

2.1 Seed Preparation and Sterilization

The sunflower seeds soaked in a water bath on ± 45°C for 24 hours to break down the seed dormancy. To avoid the contamination, it sterilized on serial sterilization liquid[10]. The dry and sterilized seeds than inoculated into sterile water agar to test contaminant percentage. The seeds of peanut and local varieties soybean used for the control treatment.

2.2 Inoculation of active sclerotia

The sclerotia of *S. rolfsii* harvested from soil nearby infected peanut. The used sclerotia than selected with several criteria were indicated on active mode. The criteria were: its colour is tan or cream, not too dark brown or black, has the same size were measured with Scion Image ver 3.0 and their hyphae have started the initial growth maximum two days after inoculation in all used media. Each Petri dish inoculated a single selected sclerotium.

2.3 Infection Period observation

The three days inoculated sclerotia on each media: Potato dextrose agar and Water Agar, used for observation of infection periods. Ten seeds of the tested and sterilized seeds inoculated in the same Petri dish. Its placed 7 mm from the edge of the fungal colony or the tip of fungal hyphae. The observation was done on every six hours to observe the attachment hyphae to the seeds or initial root.

2.4 Sclerotia development observation

The observation of sclerotia growth was done by observing 25 random sclerotia in each petri dish on each treatment. The observation begins after appear the hyphae mass on the medium surface or near growing seeds. Then the observation continued every 6 hours to monitor the development time of sclerotia in each media and seeds treatment.

2.5 Sclerotia size observation

The observation of sclerotial size was done by observing random 25 random sclerotia in each petri dish on each media and seed treatment. The observation begins after eleven days after inoculation. The sclerotia size observed on the binocular microscope and measured with Scion Image ver 3.0. The sclerotia size data on each media and seeds treatment than compared to each other.

2.6 Post analysis

All data form the observation than tested with analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (α: 5 and 1%).

3. Results and Discussion

The first criteria for right refugia plant are not suitable as plant pathogen-host for any host, following by cultivated crop. In the phytopathological aspect, the compatibility test for a plant as refugia can be done by comparing the pathogen development on the true host and the selected plant. The plant pathogen development can be used as the variable such as the ability of plant-pathogen to infected some seeds or plant, The resulting test on each variable must compare with the true host of a selected plant pathogen.

The infection percentage of the fungal pathogen on some plant or seed is different. The plant host and viable nutrition on the environment can affect the ability of fungal pathogen on their pathogenesis process. Based on the result, the compatibility test of Sunflower IPB Bm 1 test as refugia was reflected

in similar things. At the same observation time, the fungal pathogen *S. rolfsii* can infect the sunflower seeds more than their true host, the peanut and local soybean (Table 1). The infected seed quantity was the difference between each used media. Based on media, the number of infected seeds is higher in water agar than Potato dextrose agar. The comparison result showed on IPB Bm 1, and another seed was not a significant effect on each other.

The different effect of each media illustrates the nutrition variable on the environment. Moreover, the seven-millimetres gap of colony and seed location illustrates the inoculum location and host plant in field condition. Typical saprophytic fungal will grow fast when the nutrition quantity is enough for supporting fungal growth. Its effect on fungal pretension to infect the plant host. *S. rolfsii is* known as saprophytic fungal.

Table 1. Percentage of Seeds infection

Treatment	Infected seed (%) / Media	
	PDA	Water agar
IPB Bm 1	85±0.4 ab	100 ±0.1a
Peanut Seeds	80±0.25 a	98 ± 0.5 a
Local Soybean	80±0.5 a	98 ± 0.5 a

[a,b] Means in the same column followed by different letter are significantly different (α=5%).

The little difference result was shown on the infection period. Infection period means the time needed of the fungal pathogen to attach-penetrate and colonizes plant region[11]. The result showed that *S.rolfsii* need more time to infected IPB Bm 1 than other seeds. Compared due to the control treatment, on the peanut seed and local soybean, the infection time requirement of the fungal pathogen to infected sunflower are six hours slower than other (table 2). The infection process of *S.rolfsii* generally occurring in developing root region on all used seed. The colonizing fungal hyphae in the root produce the haustoria-like formation (figure 1). It's mass of fungal hyphae but not sclerotia. That hyphal mass will continue their development to the sclerotia when it develops on the outer root surface.

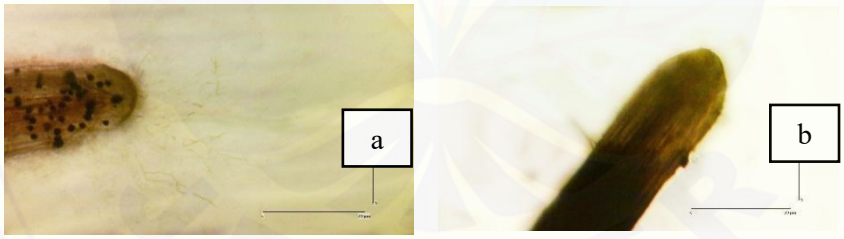


Figure 1. The infected root tip of IPB Bm 1 (a) and healthy tip root (b)

Table 2. Infection Period

Treatment	Infection time (hour)/Media	
	PDA	Water agar
IPB Bm 1	22.6±0.6 b	16.8±0,1 ab
Peanut Seeds	16.5±0.1 a	12.9±0.8 a
Local Soybean	14.1±0.2 a	12.8±0,6 a

[a,b]means in the same column followed by different letter is significantly different (α=5%).

The infection period on water agar is known faster than different media. Based on [12] the fungal attach the host surface faster when lacking of nutrition growth than rich nutrition condition. Fungal need sustainability growth. and to reach that goal, fungal need to absorb available nutrition. One of available nutrition source is on the host[13]. The availability nutrition located at the host makes fungal growth-focused to reach the host surface and start their pathogenicity process on its surface.

The development stage of sclerotia until well developed on each seed and media was the difference on each other. Based on Hou *et al*[14], nutrition and host be the significant component to the fungal growth and development stage. Based on the result, the development stage from hyphae to the well-developed sclerotia in Sunflower seeds are 7,1 days faster than the control treatment and 1,2 days faster than other seeds. The development of sclerotia in the seed reflected the next stage of *S.rolfsii* growth, the dormancy stage.

The dormancy form of *S.rolfsii* in the agriculture environment is harmful[15], especially to the next planting season for several commodities. Sclerotia became primer inoculum of the stem rot disease. Several plants were attacked by the disease, like chili, cucumber peanut, soybean, vegetable, and other leguminous [16]The existence of primer inoculum caused the high probability of stem rot disease incidence than an environment without the inoculum in the future. Correlation of sclerotia development time to disease incidence not well understood. However, if sclerotia formed in the fastest way, it will accelerate the availability of disease inoculum in the field. Based on [17], sclerotia can survive and still active for two years in natural condition.

Table 3. Sclerotial Development time

Treatment	Development time (days)/ Media	
	PDA	Water agar
IPB Bm 1	6,9±0,3 b	7,8± 0,2 ab
Peanut Seeds	7,1±0,2 b	8,4±0,3 ab
Local Soybean	7,4±0,4 b	8,6±0,3 ab
Control (without seeds)	13,1±0,4 a	9,4±0,1 a

[a,b] Means in the same column followed by different letter is significantly different (α=5%).

Based on plant disease epidemiology theory, the higher quantity of inoculum can be caused the disease growth faster on the plant population at the same time[18]. The higher quantity inoculum in stem rot disease can be interpreted to the number of available scletoria, and the size of the active part where it carried on. The outer surface of sclerotia known as dead hyphae and develop to the protection layer, the center layer is active hyphae. It is mean bigger the sclerotia, and more significant active part can bring it on. Based on the result, the sclerotial size on each seed and media was different. However, the sunflower IPB Bm 1 cause the *S.rolfsii to* develop their sclerotia bigger than on other seeds or control treatment. The different of sclerotial size on each other more than 40 μm^2 (table 4).

Table 4. Average Sclerotial Size

Treatment	Sclerotia size (μm^2)/Media	
	PDA	Water agar
IPB Bm 1	204,5± 0,9 b	174,5±0,4 a
Peanut Seeds	160,7±0,6 a	160,8±0,6 ab
Local Soybean	118,7±0,30 ab	161,8± 0,5ab
Control (without seeds)	160,4±0,45 a	170± 0,3 a

[a,b] Means in the same column followed by different letter is significantly different (α=5%).

4. Conclusion

Based on the phytopathological aspect, the sunflower is can be utilized as Refugia plant but not well recommended on the legume, vegetable, or another host plant of *S.rolfsii*. It reported can well support sclerotia development compared to that true host. This result only for describing variety. The different result may occur on different variety and different pathogen. In line with that, became highly recommend for adding the phytopatologial aspect on the selecting refugia plant in the future. Aimed to reducing the possibility the refugia plant became alternate host and inoculum source of main pathogen.

5. References

[1] Purba E 2019 *Insect management with refugia plant in upland rice (Oryza sativa L.)* In: IOP Conference Series: Earth and Environmental Science IOP Publishing p 12138
[2] Anggraini E, Pardingotan R, Herlinda S, Irsan C and Harun MU.2020 *J. Appl Agric Sci Technol.* **4**(2)101–17
[3] Billah KMM 2017 *Int J Adv Agric Sci.* **2**(1)
[4] Rauf S2019 *Breeding strategies for sunflower (Helianthus annuus L.) genetic improvement. In: Advances in plant breeding strategies: industrial and food crops.* Springerp. 637–73.
[5] Brotodjojo RRR, Arochman T and Solichah C 2019 *Effect of flowering plants on population dynamics of rice stem borers and their natural enemies* In: IOP Conference Series: Earth and Environmental Science IOP Publishing p 12015
[6] Kindeya YB, Golla WN, Kebede AA and Sibhatu FB 2018 *J Biomater* **2**(2)58
[7] Gulya T, Rashid KY and Masirevic SM 1997 *Sunflower Technol Prod.* **35**263–379
[8] Fayzalla EA, El-Barougy E and El-Rayes MM 2009 *J Appl Sci.* **9**(12)2272–2279
[9] Jacob S, Sajjalaguddam RR, Kumar KVK, Varshney R and Sudini HK 2016 *J Gen Plant Pathol* **82**(2)96–104
[10] Kochman JK and Langdon PW 1986 *Aust J Exp Agric* **26**(4):489–92
[11] Pagán I and García-Arenal F2018 *Int J Mol Sci.* **19**(3)810
[12] Hartman K, van der Heijden MGA, Wittwer RA, Banerjee S, Walser J-C and Schlaeppi K2018*Microbiome* **6**(1)1–14
[13] Divon HH and Fluhr R 2007 *FEMS Microbiol Lett.* **266**(1)65–74
[14] Hou Y, Na R, Li M, Jia R, Zhou H and Jing L 2017 *J Plant Pathol.* **2017** 17–26
[15] Erental A, Dickman MB and Yarden O 2008 *Fungal Biol Rev.* **22**(1)6–16
[16] Kator L, Hosea ZY and Oche OD 2015 *Ann Biol Res.* **6**(11)78–89.
[17] Taylor A, Coventry E, Handy C, West JS, Young CS and Clarkson JP 2018 *Plant Pathol.* **67**(6)1286–1295
[18] Yuen J and Djurle A 2020 *Plant Pathol Plant Dis.* **2020** 243

Acknowledgment

We acknowledge the Islamic Development Bank- Ministry of Research, Technology and Higher Education - University of Jember Project by Overseas Non Degree Training Program 2018-2019 with Letter of Guarantee (LOG) No. 429/D4/SP/4IN1/IX/2018 and this work was supported by Research and public service bureau (LP2M) University of Jember with Contract Number 2642/UN25.3.1/LT/2020.