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Original Article

Pathogenicity of *Sclerotium rolfsii* Sacc. to several varieties of peanut on various dosages of dolomite in peatland

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Abstract

Stem rot disease caused by *Sclerotium rolfsii* Sacc. and soil acidity are the two problems that need to be addressed on peanut cultivation in peatland. Dolomite addition not only reduces soil acidity but also affect fungal pathogenicity. To study this, we used split plot design with dolomite dosages of 0, 10, and 20 t ha^{-1} as the main plots, and peanut varieties viz. Hypoma-1, Hypoma-2, Jerapah, Talam-1, Talam-2, Talam-3, Bison, and Takar-2 as the subplots. Three replications were used for these combinations. Dolomite dosages and peanut varieties interacted significantly on the pathogenicity of *S. rolfsii* at 15 DAI and filled pod percentage. The highest pathogenicity (30%) was found in Takar-2-20 t ha^{-1} dolomite, while the lowest pathogenicity (1%) was found on Hypoma-2-0 t ha^{-1} dolomite. The highest percentage of filled pods was on Bison-10 t ha^{-1} dolomite (79%), and the lowest was on Takar-2-0 t ha^{-1} dolomite (29%). Singly, the highest pathogenicity of S. *rolfsii* was found on Takar-2, while Hypoma-2, Jerapah, Talam-2, Talam-3 & Bison were more resistant. Dolomite dosage singly was only affecting the growth and the percentage of filled pods. The highest filled pods were on 10 t ha^{-1} dolomite, however if the increase to 20 t ha^{-1} dolomite had insignificant effect to enhance filled pods. These results indicated that resistant-high-yielding variety of peanut is most likely to survive stem disease and being productive in peatland enriched with proper amount of dolomite.

Keywords: Stem rot disease, *Sclerotium rolfsii*, Peatland, Acidity, Pathogenicity

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Introduction

Indonesia owns 50% or around 15-20 million hectares of the world's tropical peatlands. The 10.5 million hectares of them are cultivated, while the rest belongs to the protected areas (Najiyati et al., 2005; Jaenicke et al., 2008). With the improvement of soil acidity, fertilization, and drainage, as well as controlling weeds, pests, and diseases, the land might just be productive enough for agricultural activity. One of the potential crops that can grow in peatlands is peanut (*Arachis hypogaea* L). Some peanut varieties such Jerapah, Bison, Domba, and Gajah, could produce upto 1.77 t ha^{-1} dried pods when planted in peatland with the improvement of soil condition (Azis et al., 2013).

One problem to address is the stem rot disease caused by *Sclerotium rolfsii* Sacc that can cause death to the infected plants. The fungus produces white mycelia and abundant small-round sclerotia in vitro, and in vivo where they found at the stem base of infected plant, on the soil, and on the plant debris around it (Songvilay et al., 2013). The pathogen infects the plants at the vegetative and generative stages. The pods are brown-rot, covered with white mycelia. *S. rolfsii* stem rot spreads out to all peanut regions in Indonesia, including the neighboring countries like Malaysia, Thailand, and the Philippines (Rochjatun et al., 2011).

Some high yielding varieties released by Balitkabi (Indonesian Legume and Tuber Crops Research Institute) might have different resistance to *S. rolfsii* when planted on peatland. Varieties of Hypoma-1, Takar-1, Bima, and Talam-1 are categorized as sensitive to *S. rolfsii* since they experienced 80-90% disease incidence within the incubation period of 21-28 days (Tantawizal and Rahayu, 2017).

On the other hand, dolomite is one way to improve the alkalinity of peatlands (Shaaban et al., 2015; Pamungkas et al., 2017). The reduction of soil acidity might affect the activity of organisms in the soil, including fungal pathogens that cause peanut diseases. *S. rolfsii* can grow on wide pH range (Muthukumar and Venkatesh, 2013), ranging from acidic to alkaline, with the optimum grow at near neutral pH (Rout et al., 2015; Bhagat, 2011).

To open wider possibility to peatlands cultivation and the management of *S. rolfsii* stem rot on peanut, here we combined the treatment of dolomite dosages with several peanut varieties to see which interactions reduce the pathogenicity of *S. rolfsii* on peatland.

Material and Methods

Location, design and fungal source

The experiment was conducted on peatland located in Kuala Pesisir subdistrict, district of Nagan Raya, Aceh, at the geographic coordinate of 4°06'36.8"N 96°14'27.9"E, from April to October 2017. Split plot design (Gomez and Gomez, 1984) was used, with the dosage of dolomite viz. 0, 10, and 20 t. ha^{-1} as main plots, and the peanut varieties viz. Hypoma-1, Hypoma-2, Jerapah, Talam-1, Talam-2, Talam-3, Bison, and Takar-2 as subplots. Each treatment consisted of three replications. Duncan's test was used at 5% significance level to further confirm the result.

S. rolfsii inoculum was obtained from the stem base of the infected plant using baiting technique. The soil around the stem base was mulched with plant debris to maintain the moisture and to induce fungal grow. After three days the mulch was opened to see whether the white mycelia and the round, white to dark brown fungal sclerotia had grown. Further confirmation was done microscopically to ensure that the hyphae bore clamp connection, a structure that characterized *S. rolfsii*. The fungus was grown in Potato Dextrose Agar (PDA) medium in a 100 mm in diameter Petri dish and incubated until the sclerotia were formed.

Land preparation, planting and fungal inoculation

The land was ploughed and plotted to the size of 2x3 m to make a total of 72 plots. These plots were bordered by a 30 cm wide trench. Dolomite was spread and mixed thoroughly with the soil according to the treatment. The plots were incubated for 14 days prior to planting. Each plot was planted with 100 peanut seedlings with the space of 20 x 30 cm. In each plot, 27 plants were selected and assigned as observed plants throughout the study.

The inoculation of *S. rolfsii* was carried out one week after planting. Four sclerotia were used to inoculate the seedlings. They were attached to the stem base bordered by the soil, and covered with a piece of wet cotton to stimulate the germination and to maintain humidity (Pandey et al., 2010). The chemical properties of the soil were then analyzed. Weed was manually controlled through weeding, while pests were chemically controlled whenever an incidence occurred.

The observations

The incubation period (IP) was counted as the time (d) needed for the first symptom on the infected plant to appear since the inoculation of the pathogen. The pathogenicity of *S. rolfsii* however, was defined as the ability of the fungus to cause disease, and was observed with three parameters; disease severity (DS), disease incidence (DI), and permanent wilting (PW) of the plants. DS, DI, and PW were all observed at 15, 30, dan 45 days after inoculation (DAI). [Table 1]

DS was counted according to the modified scales of Shokes et al. (1996) (Table 1), using the formula of Chiang et al. (2017):

$$DS\ (\%) = \frac{\sum(Cf \times Sr)}{(To) \times (Mi)}\ x\ 100$$

DS = Disease severity, Cf = Class frequency, Sr = score of rating class, To = Total number of observations and Mi = Maximal disease index. DI was defined as the percentage of infected plants compared with the number of healthy plants. WP was the percentage of plant underwent permanent wilting within the healthy plants.

Table-1: Severity category of peanut wilt disease caused by *S. rolfsii* (modified from Shokes et al., 1996)

Scale	Category
0	A healthy plant
1	Lesions on the stem, up to 25% of the plants symptomatic (wilted, dead, or dying)
2	26-50% of the plant symptomatic
3	Over 50% of the plant symptomatic

The growth indicators of peanut viz. the height (cm) and the number of branches, were observed at 30, 45, and 60 days after planting (DAP). Peanut production was assessed using four parameters viz. dry weight (g), pod weight (g), percentage of filled pods, and potential yield of peanuts (t ha^{-1}), which were all observed after the harvest. Dry weight was the overall dry weight of the plant per plot, from canopy to root. Pod weight per plot was the dry weight of the pods per plot. The percentage of filled pod was the number of filled pods per plot divided by the total number of pods per plot. The potential yield was counted as the average pod weight per plant multiplied by the number of plants in one hectarage.

Results

The chemical properties of peat soil

The addition of dolomite improved peat soil chemical properties like pH, exchange cation, base saturation, and electrical conductivity (Table 2). Overall, the addition of 10 t ha^{-1} dolomite resulted in a higher increase to these properties compare to the dosage of 20 t ha^{-1} dolomite. At the addition of 10 t ha^{-1} dolomite, the pH was raised from 4.11 to 5.65, and the exchange of Ca and K was brought up from 0.23 to 0.41 and 0.23 to 0.31 respectively. The exchange of Mg however, was only slightly affected. Other soil chemical properties such as exchange Ca, Mg, K, base saturation, and electrical conductivity were also raised, while the cation exchange capacity was reduced. [Table 2]

Table-2: The chemical properties of peat soil

Element of Analysis & Method	Unit	Dosage of Dolomite		
		0 t ha^{-1}	10 t ha^{-1}	20 t ha^{-1}
Soil Reaction				
pH (H_2O) (1:2.5)- Electrometric		4.11	5.65	6.25
pH (KCl) (1:2.5) - Electrometric		3.55	4.99	5.48
Exchange cation, 1N NH_4COOCH_3 pH 7:				
Exchange Ca	cmol kg^{-1}	3.74	19.23	19.85
Exchange Mg	cmol kg^{-1}	0.40	0.41	0.42
Exchange K	cmol kg^{-1}	0.23	0.31	0.73
Cation exchange capacity = CEC	cmol kg^{-1}	101.60	94.80	83.20
Base saturation	%	4.46	21.20	25.44
Electrical conductivity-EC	mS cm^{-1}	0.20	0.40	0.55

Table-3: The effect of interaction between dolomite dosage and plant varieties to the pathogenicity of *S. rolfsii* at 15 DAI on peanut planted in peatland.

Parameters (%)	Dolomite dosage (t ha^{-1})	Varieties															
		Hypoma-1		Hypoma-2		Jerapah		Talam-1		Talam-2		Talam-3		Bison		Takar-2	
Disease Severity (DS)	0	4.12	Aab	1.23	Aa	2.47	Aab	7.41	Aab	11.52	Ab	2.06	Aab	7.41	Aab	10.29	Aab
	10	13.99	Bcd	8.64	Bc	4.12	Ab	15.23	Acd	2.47	Aab	4.12	Ab	0.00	Aa	20.58	Bd
	20	20.99	Bc	11.93	Bb	7.00	Aab	10.70	Aab	6.17	Aa	4.12	Aa	10.70	Aab	30.86	Bc
Disease Incidence (DI)	0	4.94	Aab	1.23	Aa	2.47	Aab	7.41	Aab	12.35	Aab	3.70	Aab	7.41	Aab	13.58	Ab
	10	18.52	Bde	9.88	Bcd	4.94	Abc	18.52	Ade	2.47	Aab	4.94	Abc	0.00	Aa	23.46	ABe
	20	27.16	Bc	14.81	Bb	7.41	Aab	11.11	Aab	9.88	Aab	4.94	Aa	12.35	Aab	33.33	Bc
Permanent Wilting (PW)	0	2.47	Aa	1.23	Aa	2.47	Aa	7.41	Aa	11.11	Ba	1,23	Aa	6.17	Aa	8.64	Aa
	10	9.88	Bcd	6.17	Ac	0.00	Aa	8.64	Ac	2.47	ABb	0.00	Aa	0.00	Aa	14.81	ABd
	20	12.35	Bc	6.17	Abc	6.17	Abc	9.88	Ac	0.00	Aa	2.47	Aab	7.41	Ac	27.16	Bd
Filled Pod (FP)	0	62.64	Ab	50.88	Ab	50.65	Ab	58.74	Ab	50.89	Ab	52.92	Ab	62.40	Ab	29.43	Aa
	10	46.19	Aab	51.10	ABab	70.41	Bde	44.63	Aa	57.90	Abc	66.40	Bcd	78.97	Be	54.81	Babc
	20	58.59	Aa	59.36	Ba	72.11	Bb	60.69	Aa	60.56	Aa	77.88	Cb	59.17	Aa	60.09	Ba

Numbers followed by the same letter are not significant at 5% level (Duncan Test). Capital letters are read vertically, while lowercase letters are read horizontally

The interaction between dolomite dosages and varieties

The interaction between the dosage of dolomite and the varieties affected the severity of the disease, the incidence of disease as well as the percentage of filled pods at 15 DAI (Table 3). The highest pathogenicity of *S. rolfsii* was found on Takar-2, when given 10 and 20 t ha^{-1} dolomite (Table 3). On Takar-2 at 10 t ha^{-1} dolomite, DS, DI and WP were 20.58%, 23.46%, and 14.81% respectively, while on 20 t ha^{-1} these percentages were raised to 30.86%, 33.33%, and 27.16% respectively. The lowest pathogenicity at 10 t ha^{-1} dolomite was found on Bison with 0.00% on all indicators. If the dosage was enhanced to 20 t. ha^{-1}, the lowest pathogenicity was found on Talam-3 with 4.12%, 4.94%, and 2.47% on all indicators. If no dolomite was given, the highest pathogenicity was found on Talam-2 with DS, DI, and WP; 11.52%, 12.35%, and 11.11% respectively, while the lowest pathogenicity was on Hypoma-2 with 1.23% for all indicators.

The lowest percentage of filled pods was found in Takar-2 without dolomite with 29.43%, however, if given 10 or 20 t ha^{-1} dolomite, the lowest percentage of filled pods was found in Hypoma-1 with 46.19% and 58.59% respectively. The highest filled pods was on Bison - 10 t ha^{-1} dolomite with 78.97%.

Table-4: The effect of peanut varieties on the pathogenicity of *Sclerotium rolfsii*, plant growth and production, in peatland.

Parameters		Varieties															
		Hypoma-1		Hypoma-2		Jerapah		Talam-1		Talam-2		Talam-3		Bison		Takar-2	
IP		27.36	ab	30.15	bc	35.17	cd	28.67	b	30.87	bc	33.30	bc	39.75	d	22.66	a
DS	15 DAI	13.03	c	7.27	ab	4.53	a	11.11	bc	6.72	ab	3.43	a	6.04	ab	20.58	d
	30 DAI	15.09	d	11.11	bcd	5.76	a	13.20	cd	9.88	abc	7.00	ab	8.78	abc	27.57	e
	45 DAI	19.07	b	14.13	ab	9.47	a	18.52	b	12.21	ab	8.09	a	9.47	a	32.51	c
DI	15 DAI	16.05	cd	8.64	ab	4.94	a	12.35	bc	8.23	ab	4.53	a	6.58	ab	23.46	d
	30 DAI	16.05	d	11.93	bcd	6.58	a	13.99	cd	10.70	abcd	7.82	ab	9.05	abc	29.63	e
	45 DAI	20.58	c	15.64	abc	12.35	abc	19.75	bc	13.99	abc	9.47	a	10.70	ab	34.98	d
PW	15 DAI	8.23	b	4.53	ab	2.88	a	8.64	b	4.53	ab	1.23	a	4.53	ab	16.87	c
	30 DAI	13.99	d	10.29	bcd	4.94	a	11.93	cd	9.05	abcd	6.17	ab	8.64	abc	24.28	e
	45 DAI	17.70	b	12.76	ab	7.00	a	16.87	b	10.70	ab	7.00	a	8.64	a	29.22	c
BN	30 DAP	5.71	d	4.82	abc	5.35	cd	5.23	cd	4.69	abc	4.50	ab	4.32	a	5.19	bcd
	45 DAP	6.47	d	5.36	abc	5.90	bcd	5.83	bcd	5.32	abc	5.18	ab	5.08	a	5.92	cd
	60 DAP	6.58	d	5.49	ab	5.97	bcd	5.85	ab	5.39	ab	5.24	ab	5.15	a	6.06	cd
DWP		388.80	ab	392.27	ab	430.45	b	384.26	ab	413.60	b	433.70	b	432.59	b	349.18	a
WP		192.75	ab	195.38	ab	234.29	b	188.33	ab	216.09	b	237.76	b	235.98	b	154.57	a
FP		55.81	b	53.95	ab	64.39	c	54.69	ab	56.45	b	65.73	c	66.84	c	48.11	a
YP		1.14	ab	1.16	ab	1.39	b	1.12	ab	1.28	b	1.41	b	1.4	b	0.92	a

Numbers followed by the same letters in the same row not significant at 5% level (Duncan test). IP: Incubation Period (days), DS: Disease Severity (%), DI: Disease Incidence (%), PW: Permanent Wilting (%), PH : Plant Height (cm) ; BN: Branch Number per Plant ; DWP: Dry Weight of Plant per plot (g); WP: Weight of Pod per plot (g) ; FP: Filled Pods (%); YP: Yield potential ($t.ha^{-1}$) DAI= Day After Inoculation of *S. rolfsii*

When dosage was increased to 20 t ha^{-1}, the highest percentage of filled pods was in the Talam-3 with 77.88%. When dolomite was not given, the highest filled pods was found on Hypoma-1 with 62.64% (Table 3).

The effect of varieties on disease pathogenicity

Singly, the varieties significantly affect the pathogenicity of *S. rolfsii* of peanuts, as well as the growth and production of the infested peanuts (Table 4). The shortest IP was observed on Takar-2 with 22.66 d, while the longest IP was on Bison with 39.75 d. The highest DS, DI, and PW at 15, 30, and 45 DAI were found on Takar-2 with 20.58%, 23.46%, and 16.87% respectively. At 30 DAI they were 27.57%, 29.63%, and 24.28% respectively, while at 45 DAI they were 32.51%, 34.98%, and 29.22% respectively. The lowest DS, DI, and PW at 15 DAI were found on Talam-3 with 3.43%, 4.53%, and 1.23% respectively. At 30 DAI, they were observed on Jerapah with 5.76%, 6.58% and 4.94% respectively.

The highest number of branches was found on Hypoma-1 at 30, 45, and 60 DAP with 5,71, 6.47, and 6.58 respectively. Bison developed the least number of branches with 4.32, 5.08 and 5.15 respectively. The highest plant dry weight was observed on Talam-3 with 433.7 g $plot^{-1}$, while the lowest was on Takar-2 with 349.18 g $plot^{-1}$. The highest pod weight, percentage of filled pods, and yield potential was found on Bison with 235.98 g $plot^{-1}$, 66.84%, and 1.4 t ha^{-1} (Table 4).

The effect of dolomite dosage to plant growth

Dolomite dosage alone was only affecting plant height, number of branches, and percentage of filled pods. The smallest plant height, number of branches, and percentage of filled pods were found on all treatments without dolomite, either at 30, 45, or 60 DAP viz. with 14.22, 19.06, and 23.01 cm for the height, 3.94, 4.53, and 4.55 for the number of branches, and 52.32% for the percentage of filled pods. The best dosage to give the best value of all parameters was 10 t ha^{-1} either at 30, 45, or 60 DAP viz. 20.06, 27.90, and 32.55 cm for plant height, 5.48, 6.22, and 6.35 for the number of branches, and 58.86% for the percentage of filled pods. The increase of dolomite dosage from 10 t ha^{-1} to 20 t ha^{-1}, shown insignificant effect on all of these parameters (Table 5).

Table-5: The effect of dolomite dosage to the growth of stem rot infested peanut grown on peatland.

Parameters	*Dolomite dosage*		
	0 t ha^{-1}	**10 t ha^{-1}**	**20 t ha^{-1}**
Plant height 30DAP	14.22 a	20.06 b	19.90 b
45DAP	19.06 a	27.90 b	28.74 b
60DAP	23.01 a	32.55 b	37.09 b
Branch number 30DAP	3.94 a	5.48 b	5.52 b
45DAP	4.53 a	6.22 b	6.15 b
60DAP	4.55 a	6.35 b	6.25 b
Filled pods (%)	52.32 a	58.86 ab	63.56 b

Numbers followed by the same letters in the same row not significant at 5% level (Duncan test). DAP= Day After planting

Discussion

Dolomite dosages were interacted positively with peanut varieties to affect the pathogenicity of *S. rolfsii* at 15 DAI. At 30 and 45 DAI, however, the pathogenicity was no longer affected by the interaction. The different pathogenicity levels on some varieties were due to the addition of dolomite, which reduced the acidity of the peat soil. The pH was raised from 4.11 to 5.65 at the dosage of 10 t ha^{-1}, and furthermore to 6.25 at the dosage of 20 t ha^{-1} dolomite (Table 2). The fungus, *S. rolfsii,* can grow at pH range of 4-8, with the optimum growth at the pH of 6-7 (Rout et al., 2015; Ayed et al., 2018). Therefore, with or without the addition of dolomite, the pH of the peatland was actually within the growth range of the fungus.

Soil condition favoured the development of the disease, consequently, fungal pathogenicity was more influenced by the ability of the variety to resist the disease. This was shown in the interaction of dolomite dosages with varieties (Table 3) as well as the single effect of peanut varieties on pathogenicity and the production parameters of these diseased plants (Table 4). Each variety carried their own inherent resistance to the disease, which shown by the different pathogenicity level of *S. rolfsii* in infesting them (Thirumalaisamy et al., 2015; Bera et al., 2016; Rani et al., 2018). The highest pathogenicity was consistently observed on Takar-2, whenever given 10 or 20 t ha^{-1} dolomite. Even in the absence of dolomite, the pathogenicity on this variety was almost undisturbed compare to that of the highest pathogenicity, viz. Talam-2. Moreover, more mortality was observed in Takar-2 plot compare to that of Talam-2 (Fig. 1).

1 2

Fig-1: Peanut plots with (1) low (K1V5, Talam-2-10 t ha^{-1} dolomite) and (2) high (K1V8, Takar-2-10 t ha^{-1} dolomite) pathogenicity of *S. rolfsii*. Dead plants were marked with red arrows.

The dry weight of the plants and the production parameters of the infested peanuts were also influenced by varieties. Takar-2 was again among the variety to produce the lowest filled pods when given dolomite at 10 and 20 t ha^{-1}. The highest percentage of filled pods when given 10 and 20 t ha^{-1} of dolomite were found on Bison and Talam-3 respectively. Hypoma-2, Jerapah, Talam-3, Bison, and surprisingly Takar-2 were able to increase the percentage of filled pods with the addition of dolomite compared to Hypoma-1, Talam-1, and Talam-2 (Table 3).

The pathogenicity was tended to increase with the addition of dolomite, however, this addition also increased the availability of the nutrients (Table 2), thus slightly supported the plants to fill more pods (Widodo et al., 2017). Dolomite addition of 10 t ha^{-1} provided a favourable condition for the plant to grow and to fill the pods (Table 5). However, this enhancement was not seen when the dosage was added to 20 t ha^{-1}, which might be excessive to plant.

The IP coupled with DI, might be the first and the earliest indication of the susceptibility of peanut varieties to stem rot disease. In this study there were two varieties that shown the shortest IP, viz. Takar-2 and Hypoma-1, with 23 and 27 d respectively, while Bison and Jerapah were showing the longest IP of 40 and 35 d respectively (Table 3). However, amongst all varieties that employed in this study, none was infected to the degree of 80% DI and the shortest IP of 21-28 days (Tantawizal and Rahayu, 2017). Furthermore, other component of pathogenicity, viz. disease severity and wilting, as well as the production parameters indicated that all varieties could tolerate the infestation of *S. rolfsii* and able to produce up to 1.4 t ha^{-1} dried pods.

Conclusion

Dolomite dosages significantly affected the pathogenicity of *S, rolfsii* and the percentage of filled pods at 15 DAI. The highest pathogenicity was found in Takar-2 - 20 t ha^{-1} dolomite with DS, DI, and WP was 30%. The lowest pathogenicity was found in Hypoma-2 with all parameters was 1%. The lowest percentage of filled pod was found on Takar-2 without dolomite with 29%, while the highest was on Bison - 10 t ha^{-1} dolomite with almost 79%. On varieties alone, the highest pathogenicity at 45 DAI was found on Takar-2 with around 30%, whereas the varieties of Hypoma-2, Jerapah, Talam-2, Talam-3, and Bison were more resistant. The shortest incubation period was on Takar-2 with 23 d, while Bison shown the longest period with 40 d. The highest number of branches was on Hypoma-1 with 7, while the highest plant dry weight was on Talam-3 with 433.7 g $plot^{-1}$. The highest pod weight, filled pod percentage, and yield potential was on Bison, with 235.98 g $plot^{-1}$, 66.84%, dan 1.4 t ha^{-1}. Dolomite dosages however, only affected growth parameters until 60 DAP on 10 t ha^{-1} dolomite, with plant height, number of branches, filled pods, 33 cm, 5 and 59% respectively. The increase of dosage to 20 t ha^{-1} was no longer shown a significant impact.

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Contribution of Authors

Subandar I: Proposed topics and issues, conducted experiments and writing articles.
Hakim L: Contributed especially in the field of plant pathology, helped in experiments, formulate results/discussion and helped in writing articles.
Suliansyah I: Contributed in the field of plant science, especially selected peanut varieties, helped in experiments and analyze statistic and helped in writing articles
Syakur S: Contributed especially in the field of peatland management, helped in experiments and writing articles

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