

Effectiveness of the Ageratum (*Ageratum conyzoides*) Leaf Extract as Botanical Fungicide Against Twisted Disease of Shallot

*Efektivitas Ekstrak Daun Ageratum (*Ageratum conyzoides*) Sebagai Fungisida Botani terhadap Penyakit Moler Bawang Merah*

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(Received: 18 September 2019, Accepted: 24 March 2020)

Citation: Rianosa R, Hartal H, Setyowati N. 2020. Effectiveness of the Ageratum (*Ageratum conyzoides*) leaf extract as botanical fungicide against moler diseases of shallot. *Jurnal Lahan Suboptimal: Journal of Suboptimal Lands* 9(1): 1-10.

ABSTRAK

Penyakit moler merupakan penyakit utama yang dapat menurunkan kualitas dan kuantitas produksi bawang merah. Aplikasi fungisida sintetik terbukti efektif untuk mengendalikan penyakit ini, namun penggunaan dalam jangka panjang dapat meracuni organisme non-target dan merusak lingkungan. Daun bandotan (*Ageratum conyzoides*) yang dianggap gulma ternyata berpotensi sebagai anti jamur, anti bakteri bahkan anti kanker. Oleh karena itu, penelitian ini bertujuan untuk menentukan konsentrasi ekstrak daun bandotan yang paling efektif dalam mengendalikan penyakit moler pada bawang merah secara *in vivo* serta membandingkan efektivitasnya dengan fungisida sintetik. Penelitian menggunakan Rancangan Acak Lengkap dengan faktor tunggal yang terdiri dari 5 perlakuan fungisida nabati ekstrak daun bandotan (konsentrasi 1% sampai 5%), 2 perlakuan fungisida sintetik (mankozebe dan metil tiofanat) sebagai pembandingan serta 1 perlakuan kontrol (tanpa fungisida dengan media tanam steril). Ekstrak daun bandotan didapatkan dengan metode maserasi menggunakan etanol 96%. Aplikasi fungisida hanya dilakukan sekali pada umbi bawang merah sebelum tanam. Hasil penelitian menunjukkan, fungisida nabati dari ekstrak daun bandotan efektif dalam mengendalikan penyakit moler pada bawang merah. Konsentrasi 4% ekstrak daun bandotan memiliki efektivitas yang sebanding dengan fungisida mankozeb dalam mengendalikan penyakit moler serta mampu meningkatkan hasil tanaman bawang merah. Dengan demikian ekstrak daun bandotan dapat dijadikan pengganti fungisida sintetik dalam mengendalikan penyakit moler pada bawang merah.

Kata kunci: bandotan, fungisida nabati, fungisida sintetik, gulma, penyakit moler

ABSTRACT

Twisted is a major disease of onion caused by *Fusarium oxysporum* f. sp. cepae. Application of synthetic fungicides in the long-term has a negative effect to the environment. Weed leaves of *Ageratum* (*Ageratum conyzoides*) have potential to be an anti-fungal, anti-bacterial as well as anti-cancer. This study aimed to determine the best concentration of *Ageratum* leaf extract to control twisted disease and to compare the

effectiveness of botanical fungicide and synthetic fungicide. The experiment was consists of five Ageratum leaf extract concentrations as a botanical fungicide (i.e. 1%, 2%, 3%, 4%, and 5%), and synthetic fungicide namely mancozeb and metil tiofanat and control treatment (in sterile soil with no fungicide treatment). The experiment used Completely Randomized Design (CRD) with a single factor. Ageratum leaf extract was obtained through the maceration method using ethanol 96%. The application of fungicide was carried out on shallot bulbs before planting. The results showed botanical fungicides from Ageratum leaf extract were effective against twisted disease of shallots, concentration 4% of Ageratum leaf extract resulted in a longer incubation period and lower disease intensity compared to other concentrations, and the effectiveness of Ageratum leaf extract at a concentration of 4% was comparable to that of mancozeb to control twisted disease. Application of both synthetic and botanical fungicide resulted in a higher yield of shallot. Therefore, Ageratum leaf extract has potential as a substitution of synthetic fungicides to control twisted disease of shallots.

Keywords: Ageratum, biopesticide, disease, *Fusarium oxysporum*, weed

INTRODUCTION

Shallot (*Allium ascalonicum* L.) is one of the leading vegetable commodities making an important contribution to the economic development of the region in Indonesia. This commodity is used for daily consumption and even become an industrial raw material (Badan Litbang Pertanian, 2005). The data of the Badan Pusat Statistik (2017) showed that the shallot productivity in Indonesia decreased by 3.93%. The 2015 shallot production of 10.07 tons/ha declined to 9.64 tons/ha in 2016. On the other hand, the productivity of shallots in Bengkulu Province increased by 14.3%; in 2015 the production increased from 5.12 tons/ha to 5.84 tons/ha in 2016. However, its productivity only fulfilled half of the national productivity. Consequently, an evaluation of the development of shallots is needed because this commodity was a national priority in 2018 and Indonesia is projected as the main exporter of shallots in ASEAN in 2045 (Direktur Jenderal Hortikultura, 2017). One of the main causes of the decrease of shallot productivity in Indonesia is the high attack of plant-disturbing organisms (PDO). Twisted disease is one of the main diseases in shallot cultivation caused by *Fusarium oxysporum* f.sp. *cepae*. Dean et al. (2012) states that *F. oxysporum* is the number 5 cause of damage in the world in agricultural

cultivation which causes significant losses for farmers. *F.oxysporum* is a soil borne pathogen that can last a long time in the soil as saprophytes capable of infecting young plants from the field to the storage in warehouses by direct penetration or through wounds to roots and tubers (Michielse and Rep, 2009; Hasanuddin and Rosmayati, 2013). The damage of shallots due to this pathogen can reduce the economic value of shallot both in quality and quantity.

Shallot twisted disease is very difficult to control and prevention is needed from the beginning of planting. Application of synthetic fungicides on tubers before planting (seed treatment) has proven to be effective in controlling pathogens carried on seedlings and protecting them from soil-borne pathogens (Agrios, 1996) but long-term use can cause environmental damage and contaminate non-target organisms with poison. In addition, some pathogens in agricultural land are resistant to synthetic fungicides; therefore, their development is difficult to control. There have to be other alternatives needed to control them. Vegetable fungicides are one of the promising solutions because their main ingredients are easily obtainable in nature. Allelopathy derived from the plant secondary metabolites is known to play an important role as plant protection such as anti-bacterial, anti-viral, anti-fungal, and plant-based insecticides (Hajlaoui et al.,

2009). The active substances contained in the plant allelopathy can be synthesized or used in extract form. Plant extracts are easily degraded quickly in the soil thereby reducing the impact on the environment and effective support for sustainable agriculture (Cho *et al.*, 2006). *Ageratum conyzoides* which belongs to the family Asteraceae is a weed that has the potential to produce allelopathy.

The allelopathy contained in *Ageratum* consists of alkaloids, flavonoids, chomenes, benzofuran, and terpenoids (Okunade, 2002). According to Xuan *et al.* (2004), extracts from *Ageratum* can control several pathogens in rice plants, it even has the potential to be anti-cancer (Adetutu *et al.*, 2012). *Ageratum* extract also shows effectiveness comparable to the 50% iprodion fungicide in suppressing the severity of anthracnose in bananas (Arie *et al.*, 2015). Therefore, this study aimed to find out the concentration of the most effective *Ageratum* leaf extract to control twisted disease in shallots and then compare the effectiveness of vegetable fungicides with synthetic fungicides in controlling twisted disease in shallot.

MATERIALS AND METHODS

The study was conducted at the Plant Protection Laboratory, Faculty of Agriculture, Bengkulu University and the green house in Medan Baru, Muara Bangkahulu District, Bengkulu City. The study used a Completely Randomized Design (CRD) with 8 treatments and 4 replications. In total there were 32 experimental units. The treatments consisted of 5 vegetable fungicide treatments (1%, 2%, 3%, 4% and 5% of *Ageratum* leaf extract), 2 synthetic fungicide treatments (mankozeb and methyl thiophanate) and control (without fungicide with sterile growing media). *Ageratum* leaves used in the study derived from the Experimental Farm of the Faculty of Agriculture, Bengkulu University.

The *Ageratum* leaf extraction process was carried out by maceration method using ethanol 96% for 48 hours. The maceration results in the form of filtrate were obtained by filtering using filter paper, then the collected filtrate was evaporated with a rotary vacuum evaporator at 40°C so that *Ageratum* leaf extract was obtained. Concentration of vegetable fungicide was obtained by dissolving 0.5g; 1g; 1.5g; 2g and 2.5g of *Ageratum* leaf extract into sterile distilled water to a volume of 50 ml each to obtain a concentration of *Ageratum* extracts of 1%, 2%, 3%, 4% and 5%. Bauji shallot varieties and planting media in this study derived from the UPTD Balai Benih Hortikultura (BBH) in Kepahiang District of Bengkulu Province, which were suspected to have been contaminated with *F. oxysporum*.

The planting media consisted of a mixture of soil and cow manure with a weight ratio of 2:1. Specifically the control treatment used planting media sterilized using autoclaves at 121°C with a pressure of 2 atm for 30 minutes. The day before planting and the application of fungicides, the upper third of the bulbs of the shallot was cut so that the growth was uniform. The application of vegetable fungicide was conducted by soaking the shallot tubers for 1 hour in 50 ml of *Ageratum* leaf extract solution based on the treatment added with 2% Tween 80 adhesive (v/v).

The synthetic fungicide application was carried out by soaking tubers in a pesticide solution at a dose of 2 g/L for the past 5 minutes. After being soaked, the tubers were aerated for 2 hours in a clean place at room temperature. The application of this fungicide was only done once before the seedlings were planted. After the application of fungicide, the shallots were planted into a 30 x 30 cm polybag containing 5 kg of growing media in each polybag. The shallots were harvested on the 66 days after planting.

Table 1. Scale of the disease severity

Score	Severity
0	Apparently infection –free;
1	Trace - 25% leaf area infected;
2	26% - 50% leaf area infected;
3	51% - 75% leaf area infected;
4	> 75% leaf area infected;
5	Plant dead

Source: Bock *et al.*, 2010

Table 2. Analysis of variance (ANOVA) of all variable tested

Variable	F-value	F -table
Incubation period	0,65 ns	2,42
Disease incidence	1,1 ns	2,42
Percent severity index	3,5 **	2,42
Plant height	10,65 **	2,42
Number of leaves	1,32 ns	2,42
Wet weight crown	1,86 ns	2,42
Dry weight crown	1,57 ns	2,42
Wet weight bulb	2,96 *	2,42
Dry weight bulb	3,38 *	2,42
Number of bulb	1,38 ns	2,42
Bulb size	2,47 *	2,42

Remarks: ns = not significant, * = significantly different, ** = highly significantly different

The observations were made on the variables of disease, growth, and yield of shallots. The disease variables consisted of incubation period (days), DI (Disease Incidence) (%) and PSI (Percent Severity Index) (%). Growth and yield variables consisted of plant height (cm), number of leaves, crown fresh weight (g), bulb fresh weight (g), number of bulb, bulb size (g/bulb), crown dry weight (g) and bulb dry weight (g).

$$DI = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100\%$$

$$PSI = \frac{\sum(n \times r)}{R \times T} \times 100\%$$

Remarks:

DI = Disease Incidence, PSI = Percent Severity Index, n = number of infected leaf at the score, r = score (Table 1), R = maximum score on scale dan T = total number of leaf

The collected data were statistically analyzed using analysis of variance (F-test) at a level (α) of 5% using the statistical software CoStat. The significantly different

and highly significantly different results were further tested with Duncan's Multiple Range Test (DMRT) of 5%.

RESULTS AND DISCUSSION

Research Overview

The study was conducted at Medan Baru Green House, Bangkahulu Subdistrict, Bengkulu City at an altitude of 7 m above sea level with soil temperatures between 25°C - 35°C, soil pH ranged from 6.0 to 7.0 and soil moisture ranged from 70% to 90%. Rahayu and Berlian (2000) and Susanti *et al.* (2016) stated that the environmental conditions were in accordance with the conditions for growing shallots and the development of isolates of *F. oxysporum* f.sp. *cepae* of the Bauji variety shallot plant so that this research could run well until the harvest. All shallot plants got twisted disease, including the control plants. Previously, it was hoped that the control plants would not be attacked by the twisted disease because they were planted on sterile growing media. This attack still occurred allegedly due to pathogens carried on the shallot seedlings from the previous planting (seed born) (Artati, 1999; Bernadip *et al.*, 2015). This proves that the land used for shallot cultivation in BBH in Kepahiang District was contaminated by the pathogen *F. oxysporum* that the seeds carried it with them. The results of the analysis of all variables are presented (Table 2).

Fungicide Efficacy on Twisted Disease in Shallots

The observation of disease variables on shallot plants was incubation period, percentage of attack and intensity of attack. The incubation period of twisted shallot began to be observed from the first day of planting until the plants showed symptoms that were characterized by pale green leaves and curves (twisted) (Patil *et al.*, 2018). In the first week, all plants did not show the symptoms of twisted disease. Table 3 shows that the fastest incubation period

occurred in the treatment of plant fungicides of 1% Ageratum leaf extract on the 16.25 days after planting. The longest incubation period occurred in the treatment of plant fungicides of 4% Ageratum leaf extract and control plants on the 19.75 days after planting. These results were in line with the study of Wiyatiningsih (2010) that the incubation period of the fastest twisted disease in Bauji varieties shallot without fungicide treatment ranged from the 12.7-21 days after planting. The length of the incubation period in the 4% Ageratum leaf extract fungicide treatment on shallot shows that this treatment is the best in inhibiting the development of twisted disease although the results of the analysis between treatments were not significantly different.

The percentage of disease attack was observed to find out the percentage of the number of plants affected by twisted disease of the total shallot plants without looking at the severity of the disease. Table 3 shows that the percentage of attacks on 2 WAP only occurred in the treatment of vegetable fungicides of 1% Ageratum leaf extract by 25% or about 3% of the entire plant observed. However, the percentage of twisted disease attacks increased very drastically on 3 WAP, which was equal to 81% and finally all plants showed early symptoms of twisted disease at 4 WAP.

The intensity of the attack was observed to find out the severity of twisted disease on shallots. Table 3 shows that the higher the concentration of vegetable fungicide of

Ageratum leaf extract is, the lower the intensity of twisted disease shallots will be. At a concentration of 2%-5% Ageratum leaf extract was significantly different when compared to the control treatment and 1% Ageratum leaf extract. In addition, the plant-based fungicides were better when compared to mankozeb and methyl thiophanate synthetic fungicides in inhibiting the attack of twisted disease, although not statistically significantly different.

Fungicides Efficacy on Shallots Growth and Yield

Plant height, number of leaves, and crown weight are the most important aspects to measure the growth of shallot because they are able to indicate the results of photosynthates in plants which will later be translocated to the important parts of the plant especially for the formation of shallot bulb (Elisabeth *et al.*, 2013).

Table 4 shows that all shallot plants treated with fungicides (both plant and synthetic fungicides) had plant heights ranging from 40.25 cm to 43.25 cm, significantly different compared to that of the control plants (28.5 cm). The number of leaves was in accordance with the description of shallots varieties, which were 35 cm - 43 cm (Baswarsiati *et al.*, 2013). The results of this study indicate that the disease- controlled plants resulted in the higher plants height.

Table 3. Effects of fungicide on twisted diseases incidence of shallot

Treatment	Incubation Period (DAP)	Disease Incidence (%)			PSI (%)
		2 WAP	3 WAP	4 WAP	
1% Ageratum extract	16,25	25	100	100	33,00 _a
2% Ageratum extract	18,50	0	100	100	19,75 _b
3% Ageratum extract	18,75	0	50	100	18,00 _b
4% Ageratum extract	19,75	0	50	100	17,75 _b
5% Ageratum extract	19,00	0	100	100	16,50 _b
Mankozeb	18,25	0	100	100	24,00 _{ab}
Metil tiofanat	19,00	0	75	100	22,25 _{ab}
Control	19,75	0	75	100	32,00 _a
Total		3%	81%	100%	

Remarks: Numbers followed by the same letter in the same columns were not significantly different ($\alpha = 5\%$). DAP = day after planting, WAP = week after planting, PSI = Percent Severity Index

The control treatment resulted in the lower plants height. This was due to the tip of the shallot leaf withered and dried. This symptom is an advanced symptom of twisted disease thus significantly reducing plant height (Patil *et al.*, 2018). This is consistent with the results of the research conducted by Naguleswaran *et al.* (2014) that twisted caused by *Fusarium* can make the end of the plant experience necrosis. Lestiyani *et al.* (2016) also report that the diseases caused by *Fusarium* spp. can cause three symptoms of shallot plants in the form of wilting, twisted, and root decay.

Although there were no significant differences among the treatments, the leaves number of shallots showed good results, ranging from 43-74 strands/clump (Table 4). There were also no significantly different on the crown fresh weight and crown dry weight of shallot. This results

showed that the growth of shallots was still going well even though being attacked by twisted disease. When the plants grow well, the photosynthesis activity in the leaves will run normally so that it can produce photosynthates better which will be reflected from the plant yield variables.

The main purpose of agricultural cultivation is to get good and economically valuable crops. The tuber yield is the most economical part and is as an indicator of the success of shallot cultivation. The fresh weight of shallots indicates that the plants still contain water in its tissues while the dry weight indicates the photosynthetic results produced by the plant during its growth. The accumulation of dry matter reflects the ability of plants to bind energy from the sunlight through the process of photosynthesis, as well as their interactions with other environmental factors.

Table 4. Effects fungicide on the growth and yield of shallot

Treatment	Plant Height (cm)	Number of Leaves	Crown Fresh Weight (g/Clump)	Crown Dry Weight (g/Clump)
1% Ageratum extract	40,25 _a	56,5	32,78	3,93
2% Ageratum extract	43,25 _a	55,5	38,17	4,58
3% Ageratum extract	41,25 _a	65,5	33,45	4,18
4% Ageratum extract	42,00 _a	52,5	47,67	6,20
5% Ageratum extract	42,75 _a	49,0	47,84	5,58
Mankozeb	40,25 _a	74,0	52,25	6,79
Metil tiofanat	41,50 _a	43,0	33,96	3,91
Control	28,50 _b	53,0	19,69	2,36

Remarks: Numbers followed by the same letter in the same columns were not significantly different ($\alpha = 5\%$)

Table 5. Effects fungicide on the yield of shallot

Treatment	Bulb FW Weight (g/Clump)	Bulb DW (g/Clump)	Number of Bulb	Bulb Size (g/Bulb)
1% Ageratum extract	51,67 _{ab}	7,57 _{bc}	6,00	8,36 _a
2% Ageratum extract	43,85 _b	7,92 _{bc}	5,50	8,33 _a
3% Ageratum extract	35,29 _{bc}	5,96 _{bc}	8,25	6,59 _{ab}
4% Ageratum extract	53,66 _{ab}	12,03 _{ab}	6,75	8,64 _a
5% Ageratum extract	52,91 _{ab}	9,91 _{ab}	5,00	10,85 _a
Mankozeb	67,47 _a	15,11 _a	10,50	6,70 _{ab}
Metil tiofanat	41,90 _b	6,71 _{bc}	6,00	7,45 _a
Control	15,90 _c	1,89 _c	6,75	2,32 _b

Remarks: FW: Fresh Weight, DW: Dry Weight Numbers followed by the same letter in the same columns were not significantly different ($\alpha = 5\%$)

Table 5 shows that there are significant differences in bulb weights among the treatments. The best bulb weights in the plant-based fungicide treatment were produced by the treatment of 4% extract (FW= 53.66 g/clump and DW= 12.03 g/clump) which were not significantly different when compared to the synthetic fungicide mankozeb treatment (FW= 67.47 g/clump and DW= 15.11 g/clump) and methyl thiophyanate (FW= 41.90 g/clump and DW= 6.71 g/clump). Therefore, the application of vegetable fungicide *Ageratum* leaf extract can replace synthetic fungicides while increasing the yield of shallots. Looking at the number of bulb, the mankozeb fungicide treatment produced the highest number of bulb (10.50) while the number of bulb in the 4% treatment of *Ageratum* extract was the same as the control treatment (6.75). These results indicated that the number of shallots produced per clump from each treatment was not the same. In the control treatment, although the number of bulb produced was quite large, the weight was low, indicating that the size of the bulb was small. In addition, the shallot bulb in the control plants that had twisted disease was paler when compared to those of the fungicide treatment. Conversely, in the treatment of 4% *Ageratum* leaf extract, the number of bulb produced was the same compared to the control plants but the weight was heavier, indicate a larger tuber size. Both of these phenomena were related to the plant health and the distribution of photosynthates resulting from the photosynthesis.

Discussion

In general, *Ageratum* extracts effectively control the twisted disease. The 4% treatment of *Ageratum* leaf extract is able to produce a longer incubation period, lower attack intensity and better yields than other vegetable fungicide treatments. In addition, the treatment of plant-based fungicides has efficacy that was comparable to the treatment of synthetic fungicides. *Ageratum*

contains secondary metabolites such as terpenes, sterols, flavonoids, alkaloids, benzofuran, chromen, chromon, coumarin, essential oils, and tannins so that these plants are believed to have many benefits and one of them is as an anti-microbial (Kamboj and Saluja, 2008).

The ability of *Ageratum* leaf extract to control twisted disease is the one which is strongly correlated with high flavonoid content in the leaves. Mohamed *et al.* (2017) state that the higher the level of flavonoids is, the more effective it is in reducing the growth of *Fusarium* mycelium. This is because flavonoids are able to change the structure of the *Fusarium* so that they are unable to attack plants properly. Javed and Bashir (2012) also reported that 6% of *Ageratum* leaf extract was able to reduce 78% of mushroom mass so that it was effective in controlling *Fusarium solani*.

The results of the research conducted by Sugito *et al.* (2010) showed that the use of plant-based fungicides could reduce the pathogen population of *F. oxysporum* without any negative impact on the growth of tomato plants. Therefore, the use of vegetable materials can save the cost of crop production to increase the value of agricultural products (Amoabeng *et al.*, 2014). Although inexpensive and easily applied, the plant-based fungicides need to be applied periodically or repeatedly to get maximum results because the plant-based fungicides are derived from the plant materials which are easily degraded and quickly evaporate (Asikin, 2016). In general, mankozeb synthetic fungicides are effective in controlling twisted disease compared to other treatments. In fact, according to Patil and Nargund (2017), mankozeb is one of the most effective synthetic fungicides in controlling the disease of twisted shallot. This is because 80% of mankozeb consists of 62% dicio-carbamate, 16% manganese, and 2% zinc. In addition to being able to control pathogenic fungi, mankozeb is able to increase nutrient fulfillment for plants so

that they can grow optimally despite having the disease. Furthermore, mankozeb is also able to survive for 3 months before being degraded so that it can protect the tubers longer from the disease (Doneche *et al.*, 1983). However, the use of mankozeb can reduce the population of soil fungi, especially antagonistic agents which results in the loss of the balance of soil ecosystems making it easier for resistant pathogens to attack plants (Sari *et al.*, 2014; Lestari *et al.*, 2018).

On the other hand, although methyl thiophanate is a systemic synthetic fungicide, its effect is no more effective than mankozeb fungicide in controlling twisted disease. This is because mankozeb is a contact fungicide that is able to inhibit the early development of spores on the surface of shallot bulb so that it protects the shallot faster than methyl thiophanate which is a systemic fungicide that takes longer to protect plants. In line with the study conducted by Vengadaramana and Costa (2014), although effective, the application of the methyl thiophanate fungicide which is carried out only once before planting in shallot tubers is insufficient to control the twisted disease as a whole.

In the control plants, almost all observed variables produced the lowest values except the number of leaves and tubers. Although the number of leaves and tubers produced was equivalent to other treatments, it still produced the lowest crown weight and the smallest tuber size. This is because *F. oxysporum* secretes several extracellular cellulolytic and pectinolytic enzymes after infection causing cell damage to shallot. The infected shallot tubers show disintegration of the epidermal layer and cortex tissue to make the plant metabolism disrupted (Malathi, 2013). In addition, Alberto (2014) reveals that twisted disease can cause a decrease in protein, sugar and phenol content so that it can decrease the resistance of the shallot. This is what causes the tuber size to be smaller and paler than those that receive fungicide treatment.

CONCLUSION

Botanical fungicide from *Ageratum* leaf extract was effective in controlling twisted disease in shallots. The 4% concentration of *Ageratum* extract is the best plant-based fungicide treatment resulting in a longer incubation period and low intensity of disease attack as well as resulting in canopy fresh weight, tuber fresh weight, and tuber dry weight higher than other concentrations. *Ageratum* leaf extract at a concentration of 4% has ability comparable to the mankozeb fungicide in controlling twisted disease and is able to increase the yield of shallots. Thus the *Ageratum* leaf extract can be used as a substitute for synthetic fungicides in controlling twisted disease in shallots.

ACKNOWLEDGEMENT

Our gratitude goes to the *UPTD Balai Benih Hortikultura* (BBH) of Kepahiang District, Bengkulu Province, which has facilitated researchers in the provision of Bauji variety shallot bulbs and planting media.

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