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Pengajuan Kenaikan Jabatan
dari Lektor Kepala ke Guru Besar

Oleh

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Uniersitas Bengkulu

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1. BIODIVERSITAS, JOURNAL OF BIOLOGICAL DIVERSITY (Q3, SJR = 0,33)

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Nor Liza <smujo.id@gmail.com> 1 November 2019 pukul 14.50
Balas Ke: Nor Liza <sectioneditor2@smujo.id>
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Marlin Marlin:

I believe that you would serve as an excellent reviewer of the manuscript, "Investigation to the causal agent of “Moler disease” in Indonesian shallots revealed new strains of pathogenic Fusarium oxysporum f. sp cepae," which has been submitted to Biodiversitas Journal of Biological Diversity. The submission's abstract is inserted below, and I hope that you will consider undertaking this important task for us.

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"Investigation to the causal agent of “Moler disease” in Indonesian shallots revealed new strains of pathogenic Fusarium oxysporum f. sp cepae"

Abstract. *Fusarium oxysporum* f. sp. *cepae (FOC)* is one of the special formae of *F. oxysporum* which reported to attack shallots and cause basal rot and leaf twisting disease in Indonesia. However, to discriminate of *F. oxysporum* forma speciales is uneasy, and often managed through laborous and time-intensive disease assays. Molecular approaches using virulence genes to identify fungal plant pathogens has proven successful in the past for other *Fusarium* species. This study aimed to characterize the isolates of *FOC* from various shallots in Indonesia based on their

effectors gene : *SIX3, SIX5, SIX7, SIX9, SIX10, SIX12,* SIX14, *CRX1, CRX2* and *C5*. The combined of 7 *SIX*-genes primers and 3 effector primers *C5* and *CRX1-2* used in the clustering analysis of 15 *FOC* isolates in this study showed that they succeeded in separating 15 *FOC* isolates into 4 groups through NTSys using UPGMA method by coefficient of similarity of 0.69. Based on phyllogeny analysis using 3 sequences of *CRX* genes, three *CRX* gene sequences showed differences in homologies. Sequences 2 and 3 were grouped into one cluster with others *CRX* genes (from *FOL* and *F proliferatum*), while the sequence 1 was grouped in a separate cluster. These results also showed that the *CRX1* and *CRX2* genes as putative effector genes are potential to use to classify the *FOC* according to their forma speciales. Based on the analysis of these gene markers, the FOC which attacks the onions in Indonesia in this study consisted of four strains.

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1 # Investigation to the causal agent of "Moler disease" in Indonesian
2 shallots revealed new strains of pathogenic *Fusarium oxysporum* f. sp
3 *cepae*

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8
9 **Abstract.** *Fusarium oxysporum* f. sp. *cepae (FOC)* is one of the special formae of *F. oxysporum* which reported to attack shallots and
10 cause basal rot and leaf twisting disease in Indonesia. However, to discriminate of *F. oxysporum* forma speciales is uneasy, and often
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20 attacks the onions in Indonesia in this study consisted of four strains.

21 **Keywords:** *CRX* gene, effector, *FOC*, *Fusarium*, phyllogeny, *SIX* gene

22 **Abbreviations:** FOC: *Fusarium oxysporum* f sp. *cepae*, SIX: Secreted In Xylem, CRX: , VCG: Vegetative Compatible Group, SAHN:
23 Sequential Agglomerative Hierarchycal Nested Cluster Analysis

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24 **Running title:** New Strains of Pathogenic *FOC* in Shallots of Indonesia

25 ## INTRODUCTION

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26 *Fusarium oxysporum* Schlecht emend. Snyder and Hansen are soil-borne fungi that are able to adapt and are found in
27 various types of soil both in the tropics, temperate, and desert areas (Agrios 2005). *F. oxysporum* strains spread as land
28 dwellers that are able to survive as saprophytes, degrade lignin and complex carbohydrates, and can survive in soil organic
29 particles. *Fusarium* wilt and root rot caused by members of the *Fusarium oxysporum* complex (FOSC) species are the
30 main obstacles to the production of horticulture plants throughout the world.
31 *F. oxysporum* can be found globally (Akagi et al. 2009) and is one of the most important pathogens included in the
32 genus *Fusarium*, which ranks 5th in the list of 10 main pathogens that have scientific and economic significance for plants
33 (Aoki T, O'Donnell 1999). *F. oxysporum* consists of more than 150 host-specific plant pathogen sub-species (Arie et al.
34 2000), known as forma specials (ff. Spp. Singular forma specialis, abrv. F. Sp.). Genome mapping and sequencing of
35 *Fusarium* species have successfully revealed the plasticity of the chromosome number of the species, which ranges from 4
36 to 17 chromosomes (Bluhm et al. 2007; Coleman et al. 2009). Every one forma specialist can cause disease in a narrow
37 range of host plant species and can be divided into racial groups or patotypes and VCG groups (Baayen et al. 2000).
38 *Fusarium oxysporum* f. sp. *cepae* is one of the special formae of *F. oxysporum* (Burgess et al. 1994) which causes basal
39 rot of onions (*Allium cepa*) (Entwistle 1990), both on garlic (*Allium sativum*) and onion (*Allium cepa* var. *ascalonicum*)
40 (Cramer 2000). This fungus was reported to attack shallots and cause leaf twisting disease in Sri Lanka (Kuruppu 1999),
41 and in Indonesia (Tondok 2001). *F. oxysporum* f. sp. *cepae* causes basal rot in onions in Iran (Rabiei-Motlagh et al. 2010)
42 and causes rot on garlic tubers (Jepson 2008). Pathogens infect the root or basal plate of the bulb. Further infections
43 usually occur at the end of the growing season, and the most severe losses are experienced in postharvest storage.
44 Some vegetative compatibility groups (VCG) in f. sp. *cepae* showed the presence of genetic variation in this forma,
45 because VCG can be considered a different clonal lineage in the population of *F. oxysporum* (Kistler 1997). This diversity
46 shows that the pathogenicity of various strains of *F. oxysporum* has evolved to onion plants. Formae speciales are often of
47 polyphyletic origin (Baayen et al. 2000), and pathogenic strains may share a higher level of sequence similarity of

conserved genes with strains that are nonpathogenic or pathogenic toward another host (Kistler et al. 1997; Lieven et al. 2009). Information about the pathogenicity genes associated with the F. oxysporum will be very useful to better understand the complexity of *F. oxysporum* and can further be considered in engineering plants resistant to pathogenic *Fusarium*. However, to discriminate of *F. oxysporum* forma speciales is uneasy, and often managed through laborous and time-intensive disease assays (Recorbet et al. 2003; Covey et al. 2014; Lieven et al. 2009). Molecular detection approaches are therefore highly desired.

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Diagnostics based on genes like that encoding translation elongation factor 1-alpha (EF1 α) or the ribosomal intergenic spacer (IGS) are proven to be only useful to discriminate between fungal species (Cennis et al. 2003, Haegi et al. 2013) but often unreliable to discriminate on forma speciales (Zhang et al. 2005, Haegi et al. 2013, Lin et al. 2010). Several molecular markers based on genomic region have been developed ie. RAPD, SCAR, but the markers are suboptimal for forma specialis discrimination because they are not necessarily required for virulence and are often little to no sequence data available in public databases for comparison with other sequences. The robustness of the markers can be verified only by screening against a large collection of strains (Lieven et al. 2009).

Molecular studies on *Fusarium oxysporum* complex has shown an association between forma speciales and genes related to pathogenicity. This is very interesting, because the identification based on pathogenicity genes has not been done much on *F. oxysporum* f. sp. *cepae*. It was recently shown that host specificity is associated with the suite of effector genes present in the genomes of *F. oxysporum* strains (van Dam P et al. 2016). Both presence-absence polymorphisms and the sequence type of individual effector genes turned out to be predictive for a strain's host range. These genes therefore form the most solid base for discrimination of formae speciales within the *F. oxysporum* species complex (FOSC) (Recorbet 2003; Lieven et al. 2009). Indeed, use of virulence genes to identify fungal plant pathogens has proven successful in the past for other *Fusarium* species (Hogg et al. 2007; Mbofung et al. 2011). Within the *FOSC*, this approach has been applied to distinguish *Fusarium oxysporum* f. sp. *cubense* tropical race 4 by targeting a candidate effector gene (Aguayo *et al.* 2017). Additionally, *Fusarium oxysporum* f. sp. *lycoperici* and *F. oxysporum* f. sp. *cubense* can be discriminated from other formae speciales through the use of PCR primers designed to detect specific *Secreted In Xylem* (SIX) effector gene sequences (Van Der Does et al. 2008; Lieven et al. 2009; Fraser-Smith 2014.).

This study aimed to characterize the isolates of *Fusarium oxysporum* from various shallots in Indonesia based on the existence of genes associated with their pathogenicity (effectors) consists of *SIX3, SIX5, SIX7, SIX9, SIX10, SIX12* and SIX14. And also based on new putative effectors genes : *CRX1, CRX2* and *C5*.

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MATERIALS AND METHODS

Study area

The study was conducted at the green house and microbiology laboratory of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor (6°34'31.58"S;106°47'07.37"T, 221mabovesealevel), in 2017.

Procedur

Isolation, morphology and identification of the causal fungi

The plants were washed under running tap water. Sections of diseased tissues were surface sterilized in 70% ethanol for 30 s followed by 30 s in 0.5% (v/v) NaOCl, then rinsed in sterile distilled water and air-dried on sterile filter paper. The disinfected pieces were cut into 3–5 mm pieces, placed on *F. oxysporum* selective agar medium (Komada 1975) and incubated for 8–14 days at 25ºC. After 2 or 3 days, *Fusarium*-like colonies were observed, and hyphal tips of the colonies were transferred to potato dextrose agar (PDA). The pure cultures of the isolates were obtained using a single-spore culture technique (Leslie and Summerell 2006). Representative isolates were maintained on PDA plants. Macroscopic and microscopic characteristics of the pure cultures were studied on PDA and synthetic nutrient agar (SNA; Leslie and Summerell 2006) cultures, and the species were identified using illustrated keys (Nelson *et al.* 1983; Leslie and Summerell 2006).

Genomic DNA extraction

In order to obtain DNA from each of the identified *FOC*, single-spore from fisteen isolates of selected *FOC* were grown for five days at 25°C in Potato Dextrose Agar (PDA) (Difco). Mycelium (~0.1-0.2 g) was collected using sterile scalpel from PDA media and placed in eppendorf tubes. Culture cells were opened by adding 500 µL of CTAB extraction buffer (100 mM Tris HCl (pH 8), 2% (wt/v) CTAB, 50 mM EDTA, 0.7 M NaCl, 0.17% (v/v) β-mecarptoethanol and 1% (w/v) PVP), pre-warmed to 65°C, two glass beads added and the mixture placed in miller at a frequency of 30 sec for 5 min. Samples were incubated at 65°C for 30 min in a water bath, then extracted with phenol/chloroform/ isoamylalcohol (25:24:1) and chloroform/isoamylalcohol (24:1). DNA was then precipitated by adding two volumes of absolute ethanol and pelleted by centrifugation for 15 min at 15,0009g. The pellet was washed with 70% ethanol, air dried and resuspended

102 in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). RNA was degraded by treatment with RNase A (50 lg/ml) for 30
103 min at 37C. DNA concentration and purity was measured using Nano Drop machine.

104 *Ampification of genomic DNA using specific primers*

105 PCR amplification was carried out for 15 *Fusarium oxysporum* isolates using published primers (Tabel 1). The
106 amplification reactions were performed in 25 µL volumes in thin-walled PCR tubes after optimization in a PTC-100
107 (Programmable Thermal Controller), programmed for an initial cycle of 1 min at 95°C, 5 min at 95°C, annealing at 58°C
108 and extension 1 min at 72°C, followed by 34 cycles of 5 min at 95°C, annealing at 58°C and extension 1 min at 72°C.
109 There was a final extension step of 5 min at 72°C followed by a cooling to 4°C until samples were recovered. Amplified
110 products were analyzed on a 1.5% agarose gel in 1X TAE buffer (40 mM Tris acetate and 1.0 mM EDTA) and
111 documented using Bio-Rad documentation system.

112 Molecular characterization through the detection of the presence/absence of putative efector genes was carried out by
113 assessing all *FOC*-isolates for the presence/absence of *SIX 3,5,7,9,10,12,14 , C5, CRX1* and *CRX2*. For further
114 investigation of the variability and polymorphism between applied markers, genotyping and scoring were also conducted.
115 The data then analyzed using NTSys 2.02.

116

117 **Tabel 1.** Primers of *SIX* genes, *CRX1, CRX2* and *C5* genes

118

Primers	Sequence 5' – 3' (forward primer to reverse primer)	Annealing temp (0C)	Reference
SIX3	CCAGCCAGAAGGCCAGTTT /GGCAATTAACCACTCTGCC	51.1	Taylor et al. 2016 & this study
SIX5	ACACGCTCTACTACTCTTCA /GAAAACCTCAACGCGGCCAAA	49.65	Taylor et al. 2016 & this study
SIX7	CACTTTTTCGCCGACTTGGT /CTTAGCACCCTTGAGTAACT	48.65	Taylor et al. 2016 & this study
SIX9	GGCCCAGCCCTAGTCTAACTCC /AACTTAACATGCTGGCCGTCAATCG	53.3	Taylor et al. 2016 & this study
SIX10	GTTAGCAACTGCGAGACACTAGAA /AGCAACTTCCTTCCTCTTACTAGC	51.5	Taylor et al. 2016 & this study
SIX12	CTAACGAAGTGAAAAGAAGTCCTC /GCCTCGCTGGCAAGTATTTGTT	50.9	Taylor et al. 2016 & this study
SIX14	ACAACACCGCGACGCTAAAAAT /GCACACTCAGTGCGACAAGTTC	55.65	Taylor et al. 2016 & this study
C5	AGAGTGTGAAGTGAGGACGAGGGA /CTACGTTCGCCTCACTCATTGCCT	56.5	Taylor et al. 2016 & this study
CRX1	CACCATCTGTCTACATAAGGCCGCCC /AAAGTTCAAGGACCGGACCGCCG	58.35	Taylor et al. 2016 & this study
CRX2	TTAGTCGCACATCTACCATCACTG /GGAGTCGATCTAACTTCAGG	49.15	Taylor et al. 2016 & this study

119 *Sequencing the CRX2 gene and phylogenetic analysis*

120 For sequencing, selected three PCR amplicon sized of 400 -1000 bp generating from amplification of Isolate *FOC* with
121 *CRX2* and *CRX1* primer was extracted and purified using a QIAquick PCR Purification Kit (Qiagen). The PCR products
122 were sequenced for the DNA region coding for the *CRX2* gene using the BigDye terminator Cycle Sequencer (ABI, Foster
123 City, CA). Custal W method (Thompson et al. 1994) was used for aligning sequences. Phylogenetic tree was build using
124 MEGA version 7 (Tamura et al. 2011) from the partial sequences of the *CRX2* gene by maximum likelihood/neighbor-
125 joining method. Bootstrap values were set at 1000 replicates. The phylogenetic analysis was carried out to compare the
126 degree of genetic-relatedness of the *CRX2* gene sequences of *FOC* isolate with those available in the GenBank database.
127 Sequences obtained with each primer set were compared to GenBank nucleotide sequences by using nucleotide-nucleotide
128 Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST). Sequences were compared with closest
129 matches in GenBank through BLAST.

130 RESULT AND DISCUSSION

131 Result

132 *Isolation, morphology and identification of the causal fungi*

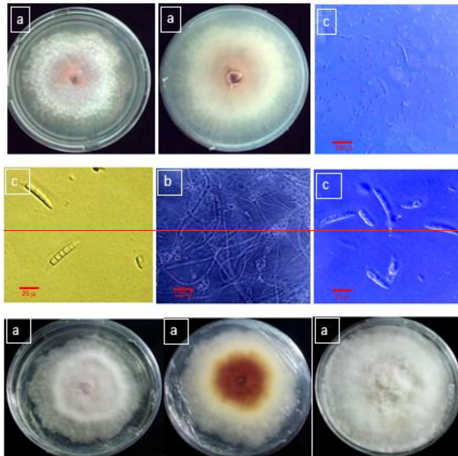
133 Thirty-five strains of *Fusarium* spp. were isolated from wilted shallot plants and bulbs from diverse field collection
134 (Table 2). All the isolates used in the present study were identified as either *F. oxysporum* (26 isolates), *F. verticillioides*

135 (2), or *F. solani* (3) and *F. proliferatum* (4) based on macro- and microscopic characteristics and were distinguished by
136 pigmentation on PDA as forming white, white pink and white violet colonies, respectively (Fig. 1 - 5).
137
138 **Table 2.** List of isolate of *Fusarium* spp. collected in this study
139

No	Species	Isolate code	Isolate originated (material or part of plant)	Host originated (scientific name)	Host variety name/site of collection
1	*Fusarium oxysporum f.sp cepae*	F.sp-1*	culture collection	*Allium cepa*	unknown/Bogor- West Java
2	*Fusarium oxysporum f.sp cepae*	F.sp-2*	culture collection	*Allium cepa*	unknown/Bogor- West Java
3	*Fusarium oxysporum f.sp cepae*	F.sp-3*	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
4	*Fusarium oxysporum f.sp cepae*	F.sp-4*	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
5	*Fusarium oxysporum f.sp cepae*	F.sp-5*	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
6	*Fusarium oxysporum f.sp cepae*	F.sp-6*	Shallot bulb	*Allium cepa*	Biru lancor/East Java
7	*Fusarium oxysporum f.sp cepae*	F.sp-7*	Shallot bulb	*Allium cepa*	Biru lancor/East Java
8	*Fusarium oxysporum f.sp cepae*	F.sp-8*	Shallot bulb	*Allium cepa*	Biru lancor/East Java
9	*Fusarium oxysporum f.sp cepae*	F.sp-9*	Shallot bulb	*Allium cepa*	Demak/ Central Java
10	*Fusarium oxysporum f.sp cepae*	F.sp-10*	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
11	*Fusarium oxysporum f.sp cepae*	F.sp-11*	Shallot bulb	*Allium cepa*	Demak/ Central Java
12	*Fusarium oxysporum f.sp cepae*	F.sp-12*	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
13	*Fusarium oxysporum f.sp cepae*	F.sp-13*	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
14	*Fusarium oxysporum f.sp cepae*	F.sp-14*	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
15	*Fusarium oxysporum f.sp cepae*	F.sp-15*	Shallot bulb	*Allium cepa*	Biru lancor/East Java
16	*Fusarium oxysporum f.sp cepae*	SP	Shallot bulb	*Allium cepa*	Super Phllips/West Java
17	*Fusarium oxysporum f.sp cepae*	KTM	Shallot bulb	*Allium cepa*	Katumi/West Java
18	*Fusarium oxysporum f.sp cepae*	1380	Shallot bulb	*Allium cepa*	unknown/Bogor- West Java
19	*Fusarium oxysporum f.sp cepae*	13793	Shallot bulb	*Allium cepa*	unknown/Bogor- West Java
20	*Fusarium oxysporum f.sp cepae*	B	Shallot bulb	*Allium cepa*	Bima Brebes/Central Java
21	*Fusarium oxysporum f.sp cepae*	BL-2	Shallot bulb	*Allium cepa*	Biru lancor/East Java
22	*Fusarium oxysporum f.sp cepae*	PKTIIB	Shallot bulb	*Allium cepa*	Pikatan/ East Java
23	*Fusarium oxysporum f.sp cepae*	AG2A2	Shallot bulb	*Allium cepa*	AG2/ West Java
24	*Fusarium oxysporum f.sp cepae*	BIO2	Shallot bulb	*Allium cepa*	Maja Cipanas/West Java
25	*Fusarium oxysporum f.sp cepae*	PKTIIA	Shallot bulb	*Allium cepa*	Pikatan/ East Java
26	*Fusarium oxysporum f.sp cepae*	PKTIIC	Shallot bulb	*Allium cepa*	Pikatan/ East Java
27	*Fusarium verticilloides*	E-1	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
28	*Fusarium verticilloides*	C-merah	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
29	*Fusarium pallidoroseum*	Bio2b	Shallot bulb	*Allium cepa*	Maja Cipanas/West Java
30	*Fusarium proliferatum*	C-kuning	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
31	*Fusarium proliferatum*	Demak-1	Shallot bulb	*Allium cepa*	Demak/Central Java\
32	*Fusarium proliferatum*	Demak-2	Shallot bulb	*Allium cepa*	Demak/Central Java\
33	*Fusarium solani*	BL	Shallot bulb	*Allium cepa*	Biru Lancor/East Java
34	*Fusarium solani*	E-3	Scallion leave	*Allium sativum*	unknown/Bogor- West Java
35	*Fusarium solani*	LGG	Shallot bulb	*Allium cepa*	unknown/Central Java

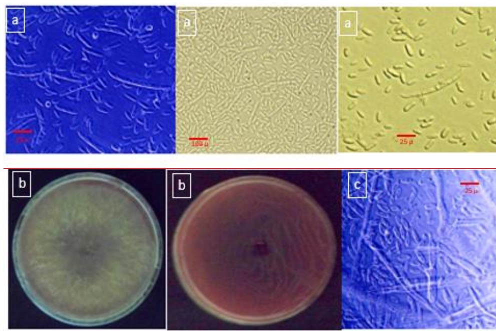
140 Note: Isolates which code followed by marked (*) are subject to use in molecular analysis
141

142



143 **Figure 1.** *Fusarium oxysporum*, (a) Colony pigmentation on PDA, (b) Chlamydospores and (c) Microconidia and macroconidia, size of
144 macroconidia and microconidia, 0.4 µm in width

145



146
147
148 **Figure 2.** *Fusarium verticillioides* (a) Microconidia and macroconidia, (b) Colony pigmentation on PDA, size of macroconidia and
149 microconidia, 0.4 µm in width (c) Monophialidic conidiophore and microconidia are abundant and. magnification for both macroconidia
150 and microconidia

151

152 *Fusarium oxysporum* isolates produced white to pale violet colonies on PDA with aerial mycelia and had a cottony or
153 somewhat ropey texture. The colour of the undersurface of the colonies among the isolates varied from pink or light to
154 dark violet or dark magenta. Microconidia were formed in false heads on short monophialides. Uni- or bicellular, and
155 ovoid to ellipsoid microconidia were abundant. Canoeshaped macroconidia with a long apical cell and a footshaped basal
156 cell formed 3–5 septa. Chlamydospores were mostly single or rarely in short chains in two-week-old cultures. On some
157 PDA cultures, macroconidia were produced from orange sporodochia.

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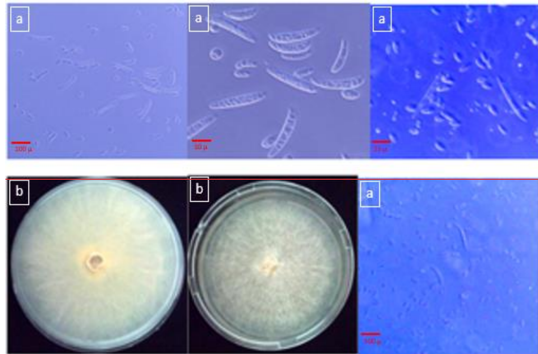

160 **Figure 3.** *Fusarium solani*. a) Microconidia and macroconidia, magnification for both macroconidia and microconidia, 0.4 µm in width,
161 (b) Colony pigmentation on PDA
162
163 *Fusarium verticillioides* colonies on PDA produced white mycelia initially and developed to dark violet pigment
164 (almost black) with age. However, some isolates had violet or pink mycelia, similar to *F. oxysporum*. Microconidia were
165 produced from unbranched monophialides in long chains in the aerial mycelium. All isolates of this species produced
166 abundant microconidia that were monocellular and oval or elliptical in shape. Macroconidia were very long, slender, bent
167 in shape, and had three to five septa with a curved or tapered apical cell. Chlamydospores were absent.
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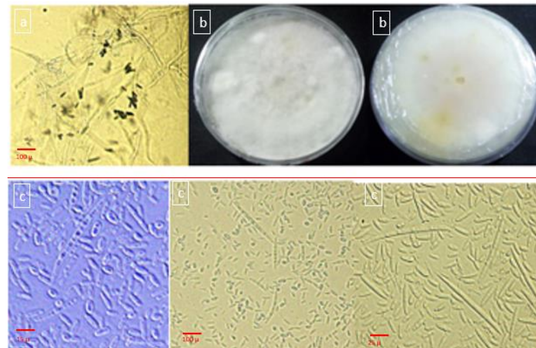

171 **Figure 4.** *Fusarium proliferatuvm.* (a) Monophialidic conidiophore and microconidia are abundant and (b) Colony pigmentation on
172 PDA, . c) Microconidia and macroconidia, size of macroconidia and microconidia, 0.4 µm in width.
173
174 *Fusarium solani* isolates on PDA formed cream- or white colonies, and in some isolates the undersurface was light
175 violet. The conidia formed on false heads on elongated phialides. The oval or elliptical microconidia were mono- or
176 bicellular. Although macroconidia were similar to those of *F. oxysporum,* they were wider than those of *F. oxysporum* and
177 had a conspicuous wall. Their apical and basal cells were round or foot-shaped and had three or five septa.
178

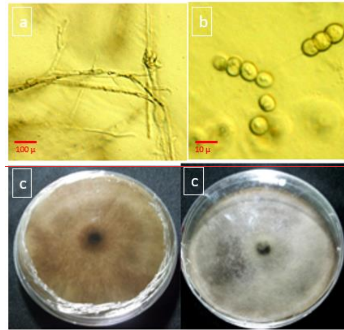

Figure 5. *Fusarium pallidosoreum*. a) Monophialidic conidiophore (b) Chlamydospore and (c) Colony pigmentation on PDA.

Ampification of genomic DNA using specific primers

A total of 7 *SIX* gene primers and 3effector primers were used for analyzing the pattern of 15 *Fusarium oxyporum* isolates from infected shallots in Indonesia (Table 3, Figure 7 and 8). The combined of 7 *SIX*-genes primers and 3 effector primers *C5* and *CRX1-2* used in the clustering analysis of 15 *FOC* isolates in this study showed that they succeeded in separating 15 *FOC* isolates into 4 groups through NTSys using UPGMA method by coefficient value 0.69. It was interesting that the isolate of *FOC* derived from scallion was separated in three different clade, i.e Clade-2, and Clade-3 (Figure 6).

Table 3. Distribution of efector gene (*SIX* genes, *C5* and *CRX* genes) in FOX isolates

Fusarium species	Host origin	Isolate code	Pathogenicity (+ / -)	SIX genes							C5	CRX1	CRX2
				3	5	7	9	10	12	14			
Fusarium oxysporum	shallot	F.sp-1	+	-	-	-	+	+	+	+	+	+	+
Fusarium oxysporum	shallot	F.sp-2	+	-	-	+	+	+	-	+	+	+	+
Fusarium oxysporum	scallion	F.sp-3	+	+	-	+	+	+	+	+	+	+	+
Fusarium oxysporum	scallion	F.sp-4	+	-	+	+	+	-	-	+	+	+	+
Fusarium oxysporum	scallion	F.sp-5	+	+	+	+	+	+	-	+	+	+	+
Fusarium oxysporum	shallot	F.sp-6	+	+	-	+	+	+	-	+	+	+	+
Fusarium oxysporum	shallot	F.sp-7	+	-	+	+	+	+	-	+	+	+	+
Fusarium oxysporum	shallot	F.sp-8	+	+	+	+	+	+	-	+	+	+	-
Fusarium oxysporum	shallot	F.sp-9	+	-	-	+	+	+	-	+	+	+	+
Fusarium oxysporum	shallot	F.sp-10	+	-	-	-	+	+	-	+	+	+	-
Fusarium oxysporum	shallot	F.sp-11	+	+	-	-	+	+	-	+	+	+	-
Fusarium oxysporum	shallot	F.sp-12	+	-	-	-	+	-	+	+	+	+	-
Fusarium oxysporum	shallot	F.sp-13	+	+	-	-	+	-	+	+	+	-	-
Fusarium oxysporum	shallot	F.sp-14	+	+	-	+	-	+	-	-	+	+	-
Fusarium oxysporum	shallot	F.sp-15	+	-	-	-	+	+	-	+	+	+	-

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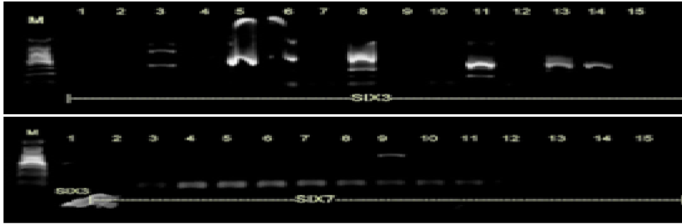
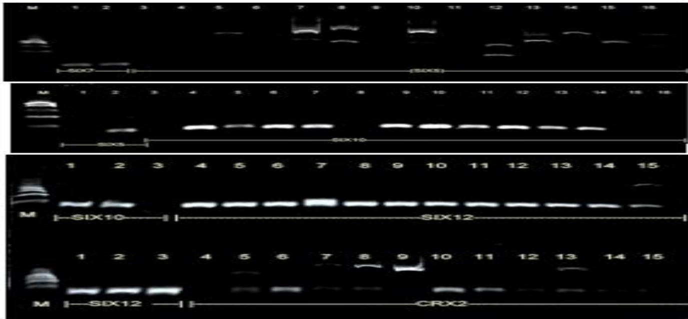
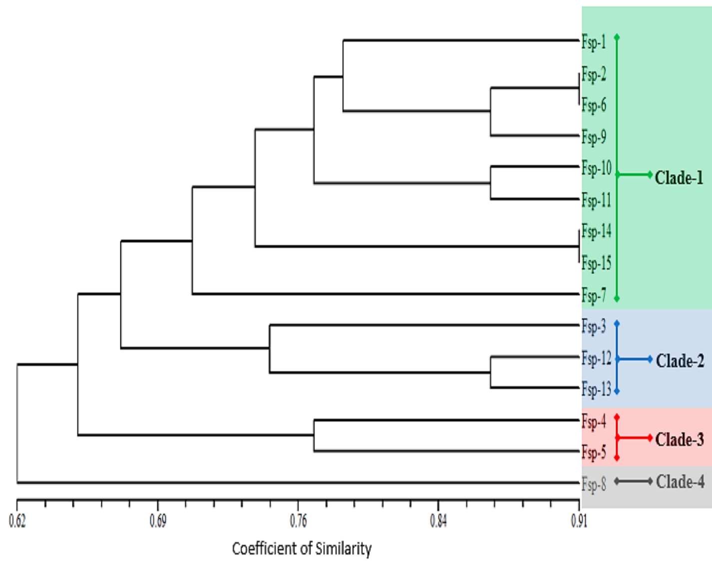


196
197
198 **Figure 7.** Amplification of DNA genomes of several *FOC* isolates with primerspecific *SIX* gene
199



200
201
202 **Figure 8.** Amplification of genomes of several *FOC* isolates with specific *CRX2* primers



203
204
205 **Figure 6.** SAHN tree of 15 *Fusarium oxysporum* isolates from bulb shallots and scellion leaves based on polymorphisms of *SIX* genes
206 and effector genes within the isolates.
207

208 *Sequencing tThe CRX2 gene and phylogenetic analysis*

209 Neighbour-joining tree of of *Fusarium oxysporumCRX1* and *CRX2* genes and their homologues, using UPGMB
210 clustering method with Kmer4_6 distance method, running on MUSCLE version 3.8.31 showed that the three *CRX* gene
211 sequences analyzed turned out to have different homologies (Figure 9). Sequences 2 and 3 were grouped into one cluster
212 with others *CRX* genes (from *FOL* and *F proliferatum*), while the sequence 1 was separated in differente cluster (Figure 9).

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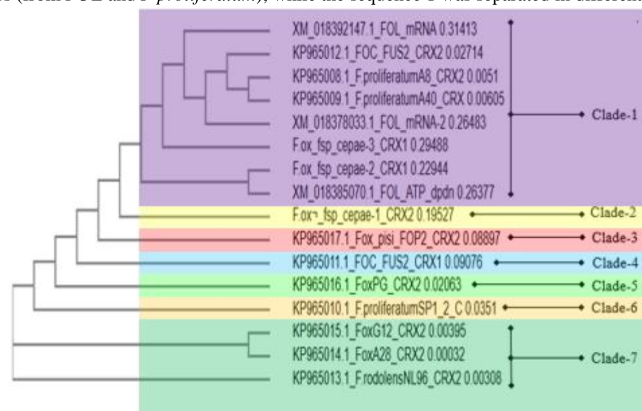

213
214
215 **Figure 9.** Neighbour-joining tree of of *Fusarium oxysporumCRX1* and *CRX2* genes and their homologues, using UPGMB clustering
216 method with Kmer4_6 distance method, running on MUSCLE version 3.8.31

217 **Discussion**

218 ~~In this study, the interesting point is that~~ the results of isolation against the source of the inoculum both in the form of
219 infected bulbs and diseased plants were found to have various *Fusarium* species i.e.: *Fusarium solani*, *F. proliferatum*, *F.*
220 *verticillioides*, *F. pallidiosoreum*. Based on the rapid test of pathogenicity using shallot bulbs, proving that the five
221 pathogens are pathogenic. This means that the five pathogens have the potential to cause disease in shallots.

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222 For many years, it has been known so far, the only causal agent of basal plate rot in shallot was *Fusarium oxysporum* f.
223 sp. *cepae*. This statement is reinforced by evidence that in this study, shallot from Demak was infected by *Fusarium*
224 *proliferatum*. This is also an indication that shallot has become a host for the pathogen. Further, there is also a high
225 probability that the cause of basal rot disease in shallots has undergone a shift, which is not only being monopoly by or
226 specific due to *F. oxysporum* f. sp. *cepae* (*FOC*), but also can be caused by other species of *Fusarium*. To confirm this
227 statement, opportunities for further research are open. The results will be very useful for determining policies and
228 strategies to control this disease in the future.

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229 ~~As comparison,~~ since 2003 it has been reported that *F proliferatum* is a pathogen in onions and garlic (Dugan et al.
230 2003; Stankovic et al. 2007; Galvan 2008) in North America. As for Italy, *F. proliferatum* has recently been reported for
231 the first time to cause rot violet basal disease in welsh onion (*Allium fistulosum*) in 2018 (Alberti et al. 2018). Although at
232 that time the status was minor-pathogen, but if it is not carefully managed, there is possibility that the pathogen becomes
233 an important one. The case is similar to have happened with *F oxysporum* f. sp. *cepa* infected shallots in Indonesia. During
234 1997 the *FOC* was known as a minor pathogen in shallot, but has only changed its status to become an important disease
235 since 2007.

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236 It has recently been shown that host specificity is associated with a series of effector genes contained in the genome
237 strain *F. oxysporum* (van Dam et al. 2016). The presence of polymorphism and the type of effector gene sequence of
238 individuals can be predicted for the range of strain hosts. Therefore this gene forms the strongest basis for discrimination
239 formae speciales in the complex species *F. oxysporum(FOSC)* (Lievens & Thomma 2005; Lievens et al. 2009). Several
240 reports indicate that the use of virulence genes to identify pathogenic fungal plants has been shown to be successful in the
241 past for other *Fusarium* species (Hogg et al. 2007). In *FOSC*, this approach has been applied to distinguish *Fusarium*
242 *oxysporum* f. sp. *tropical* race 4 cubense targeting target effector genes (Aguayo et al. 2017). In addition, *Fusarium*
243 *oxysporum* f. sp. *lycoperici* and *F. oxysporum* f. sp. *cubense* can be distinguished from other formae speciales through the
244 use of PCR primers designed to detect specific gene sequences *Secreted In the Xylem* effector gene (*SIX*) sequence (van
245 Der Do et al. 2008; Lievens et al. 2009). In this study no comparative studies were carried out for the *SIX* gene but instead
246 tested the opportunities of *CRX2* genes and *CRX1* genes as effectors which could be used as a specific species identifier of
247 *FOC*.

248 Unlike the *SIX* gene which has been known to have homologs in the form of other pathogens from *F. oxysporum* (van
249 Dam et al. 2016), there is lack information about *CRX2* and *CRX1* genes. For this, the specificity of the marker cannot be

evaluated before and cross reaction with special formae non target is found (Lievens et al. 2009). Unlike conserved core genes, virulence-related genes tend to be identical among members who have the same polyphyletic forma from *F. oxysporum* (Lievens et al. 2005; Van Der Does et al. 2008; van Dam et al. 2016). For this reason, they have a predictive value for the host strain range. Forma specialis marker is basically the smallest set of effector genes that all strains of forma specialis may have and do not exist or differ in sequence (at least one set) in all other strains (van Dam et al. 2016).The interesting thing about this study is that the *CRX1* and *CRX2* genes found in the *FOC* isolates tested had similar levels of similarity compared to those found in other *Fusarium* species.

Based on the phylogeny analysis of these genes, the *CRX1* gene from *FOC* isolates tested were more clustered with the *CRX2* gene of *F proliferatum*, as shown by isolates *FOC*-2 and *FOC*-3 on Clade 1 (Figure 7). The *CRX2* gene from isolates *FOC*-1 occupies Clade apart from the other groups. According to van der Do et al. (2008), unlike conserved core genes, genes related to virulence tend to be identical among members who have the same polyphyletic forma from *F. oxysporum.* For this reason, they have a predictive value for the host strain range. Forma specialis marker is basically the smallest set of effector genes that all strains of *forma specialis* may have and do not exist or differ in sequence (at least one set) in all other strains (van Dam et al. 2016). These results also show that the *CRX1* and *CRX2* genes at least have the potential as putative effector genes to classify the *FOC* according to their forma speciales.

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CONCLUSION

Molecular characterization through the detection of the presence/absence of putative efector genes was applied to 15 isolates of *FOC* using 7 *SIX* genes, *C5* gene, *CRX2* and *CRX1* genes. The result showed the considerable variability derived from polymorphism within the isolates in this study

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Alignment analysis between sequence from this study and sequence of gene *CRX1* and *CRX2* from ncbi database showed that the *CRX1* and *CRX2* genes as putative effector genes are potential to classify the *FOC* according to their forma speciales.

AKNOWLEDGEMENT

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Abstract

The International Diabetes Federation has recorded that in 2019 there were 463 million people with diabetes globally, and it is predicted that this will continue to grow to reach 700.1 million in 2045. Indonesia's position is included in the top 10. One way to prevent hyperglycaemia is to inhibit the enzyme α-glucosidase. Determination of the target of -glucosidase enzymes has been commonly used, and several drugs that have been developed are acarbose and voglibose. However, there are adverse effects. The active compounds of natural ingredients from plants have fewer side effects. One of the plants with antioxidant activity tested for α-glucosidase inhibition activity is Orthosiphon aristatus. However, there is still no research on metabolomics combined with chemometrics to classify the composition of compounds in plants using PCA with LC-MS/MS and looking for compounds with the most role in silico studies. After extraction and identified 86 compounds with ethanol and methanol solvents produced a PC diversity of 70.3%. In vitro results also showed that the crude extract of the O. aristatus plant was active in inhibiting α-glucosidase. in silico results prove that the best potential typical compounds are rosmarinic acid, which can be developed for further research.

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We appreciate your contribution to the quality of the work that we publish. The journal management, as mentioned before, will provide you with a 10% discount on the regular article publication fee if you submit your qualified manuscript to the journal within six months from today.

Best regards,

Nuryono Nuryono
Laboratory of Inorganic Chemistry,
Department of Chemistry,
Universitas Gadjah Mada
Phone +628156800908
Fax +62274545188
nuryono_mipa@ugm.ac.id
Editor in Chief
Indonesian Journal of Chemistry
Indexed in SCOPUS since 2012

Indonesian Journal of Chemistry
https://jurnal.ugm.ac.id/ijc
Indexed by SCOPUS since 2012

Marlin UNIB <marlin@unib.ac.id>

Certificate as reviewer

Marlin UNIB <marlin@unib.ac.id> 26 Januari 2022 pukul 22.13
Kepada: Nuryono Nuryono <nuryono_mipa@ugm.ac.id>

Prof. Dr.rer.nat. Nuryono, MS

Editor in Chief
Indonesian Journal of Chemistry

dear editor,

I have reviewed manuscript number: 71334-239170-1-RV in the Indonesian Journal of Chemistry. If you don't mind, please send me a certificate as a reviewer, because I need it for recognition of my institution. Thank you for your kind attention.

Sincerely

Dr. Marlin
Faculty of Agriculture

University of Bengkulu

Marlin UNIB <marlin@unib.ac.id>

Certificate as reviewer

Nuryono Nuryono <nuryono_mipa@ugm.ac.id> 27 Januari 2022 pukul 13.56
Kepada: Marlin UNIB <marlin@unib.ac.id>

Dear Dr. Marlin,

In the attachment, you find the certificate of reviewing you requested.

Thank you very much for your kind contribution.

best regards,

[Kutipan teks disembunyikan]
--
Prof. Dr.rer.nat. Nuryono, MS
Head of Laboratory for Inorganic Chemistry
Department of Chemistry, Universitas Gadjah Mada
Yogyakarta, INDONESIA

Editor in Chief
Indonesian Journal of Chemistry
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ISSN 1411 - 9420

Indones. J. Chem.

INDONESIAN JOURNAL OF CHEMISTRY

CERTIFICATE

OF REVIEWING

awarded January 2022 to

Dr. Marlin

In recognition of the review made for the journal

The Title of article:
PROFILING METABOLITES WITH CHEMOMETRIC ANALYSIS IN Orthosiphon aristatus EXTRACTS AS -GLUCOSIDASE INHIBITORY ACTIVITY AND IN SILICO MOLECULAR DOCKING

Editors of Indonesian Journal of Chemistry
Department of Chemistry, Universitas Gadjah Mada
Yogyakarta, Indonesia

KEPUTUSAN DEKAN FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM
UNIVERSITAS GADJAH MADA
NOMOR 2/UN1/FMIPA/KP/HK.06/2023

TENTANG

PENGANGKATAN TIM REVIEWR MAJALAH ILMIAH "*INDONESIAN JOURNAL OF CHEMISTRY*" DEPARTEMEN KIMIA
FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM
UNIVERSITAS GADJAH MADA

DEKAN FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM
UNIVERSITAS GADJAH MADA,

Menimbang : a. bahwa untuk peningkatan kualitas dan kelancaran penerbitan majalah ilmiah "*Indonesian Journal of Chemistry*" pada Departemen Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Gadjah Mada maka dipandang perlu mengangkat Tim Reviewer Inti Majalah Ilmiah "*Indonesian Journal of Chemistry*" pada Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Gadjah Mada;

b. bahwa untuk mendukung kegiatan sebagaimana dimaksud dalam huruf a, dipandang perlu mengangkat Tim Reviewer Inti Majalah Ilmiah "*Indonesian Journal of Chemistry*" pada Departemen Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Gadjah Mada;

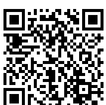
c. bahwa berdasarkan pertimbangan sebagaimana dimaksud dalam huruf a, huruf b, perlu ditetapkan Keputusan Dekan;

Mengingat : 1. Peraturan Pemerintah Nomor 67 Tahun 2013 Tentang Statuta Universitas Gadjah Mada (Lembaran Negara Republik Indonesia Tahun 2013 Nomor 165, Tambahan Lembaran Negara Republik Indonesia Nomor 5454);

2. Peraturan Majelis Wali Amanat Universitas Gadjah Mada Nomor 4/SK/MWA/2014 tentang Organisasi dan Tata Kelola (Governance) Universitas Gadjah Mada, sebagaimana telah diubah terakhir dengan Peraturan Majelis Wali Amanat Universitas Gadjah Mada Nomor 3 Tahun 2022 tentang Perubahan Keenam atas Peraturan Majelis Wali Amanat Universitas Gadjah Mada Nomor 4/SK/MWA/2014 tentang Organisasi dan Tata Kelola (Governance) Universitas Gadjah Mada;

3. Keputusan Majelis Wali Amanat Universitas Gadjah Mada Nomor 6/UN1/SK/MWA/2022 tentang Pengangkatan Rektor Universitas Gadjah Mada Periode 2022 - 2027;

4. Keputusan Rektor Universitas Gadjah Mada Nomor 6205/UN1.P/SK/HT/2021 tentang Pengangkatan Dekan Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Gadjah Mada Periode 2021 - 2026;



Dokumen ini telah ditandatangani secara elektronik menggunakan sertifikat elektronik yang diterbitkan oleh BSrE.

Memperhatikan: Surat Ketua Departemen Kimia Nomor 4/Dept.Kim/I/2023 tanggal 4-1-2023, perihal permohonan Surat Keputusan Dekan;

MEMUTUSKAN:

Menetapkan : KEPUTUSAN DEKAN TENTANG PENGANGKATAN TIM REVIEWER INTI MAJALAH ILMIAH "*INDONESIAN JOURNAL OF CHEMISTRY*" DEPARTEMEN KIMIA FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM UNIVERSITAS GADJAH MADA

KESATU : Mengangkat mereka yang namanya tersebut dalam Lampiran Keputusan ini sebagai sebagai Tim Reviewer Inti Majalah Ilmiah "*Indonesian Journal of Chemistry*" Departemen Kimia Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Gadjah Mada;

KEDUA : Tim Reviewer Inti Majalah Ilmiah "*Indonesian Journal of Chemistry*" Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Gadjah Mada sebagaimana dimaksud pada DIKTUM KESATU tersebut dalam lampiran keputusan ini;

KETIGA : Keputusan ini mulai berlaku sejak tanggal 1 Januari 2023 sampai dengan 31 Desember 2023.

Ditetapkan di Yogyakarta
pada tanggal 6 Februari 2023

Dekan,

Prof. Dr.Eng. Kuwat Triyana, M.Si.

Tembusan:
1. Ketua Departemen Kimia
2. Bendahara Pembantu Penerimaan Negara Bukan Pajak
3. Yang bersangkutan

di Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Gadjah Mada

Dokumen ini telah ditandatangani secara elektronik menggunakan sertifikat elektronik yang diterbitkan oleh BSrE.

LAMPIRAN KEPUTUSAN DEKAN FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM UNIVERSITAS GADJAH MADA
NOMOR : 2/UN1/FMIPA/KP/HK.06/2023
TANGGAL : 6 JANUARI 2023
TENTANG : PENGANGKATAN TIM REVIEWER INTI MAJALAH ILMIAH "*INDONESIAN JOURNAL OF CHEMISTRY*" DEPARTEMEN KIMIA FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM UNIVERSITAS GADJAH MADA

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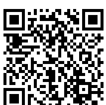
83	Lili Chrisnawati	Universitas Lampung
84	Lukman Noerochim	Institut Teknologi Sepuluh Nopember



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103	Damris Muhammad	Universitas Jambi
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105	Didah Nur Faridah	Institut Pertanian Bogor
106	Eddy Heraldy	Universitas Sebelas Maret
107	Endang Widjajanti Laksono	Universitas Negeri Yogyakarta
108	Morina Adfa	Universitas Bengkulu
109	Nurdin Ali	Universitas Syiah Kuala
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112	Tati Herlina	Universitas Padjadjaran
113	Mudasir	Universitas Gadjah Mada
114	Indra Surya	Universitas Sumatera Utara
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117	Zuchra Helwani	Universitas Riau
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119	Purwantiningsih	Institut Pertanian Bogor
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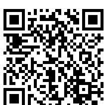
122	Rani Maharani	Universitas Padjadjaran
123	Rasi Prasetio	Badan Riset dan Inovasi Nasional
124	Retno Ariadi Lusiana	Universitas Diponegoro
125	Rindia Maharani Putri	Institut Teknologi Bandung
126	Rudi Heryanto	Institut Pertanian Bogor
127	Saharman Gea	Universitas Sumatera Utara
128	Sal Prima Yudha S	Universitas Bengkulu
129	Satya Candra Wibawa Sakti	Universitas Airlangga
130	Sayono	Universitas Muhammadiyah Semarang



Dokumen ini telah ditandatangani secara elektronik menggunakan sertifikat elektronik yang diterbitkan oleh BSrE.

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137	Suprapto	Institut Teknologi Sepuluh Nopember
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139	Titania Tjandrawati Nugroho	Universitas Riau
140	Tri Rini Nuringtyas	Universitas Gadjah Mada
141	Triana Kusumaningsih	Universitas Sebelas Maret
142	Triwindarti	Universitas Diponegoro
143	Triyono	Universitas Gadjah Mada
144	Unang Supratman	Universitas Padjadjaran
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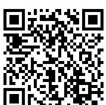
147	Waras Nurcholis	Institut Pertanian Bogor
148	Wega Trisunaryanti	Universitas Gadjah Mada
149	Welly Herumurti	Institut Teknologi Sepuluh Nopember
150	Widya Ernayati K	University of Sultan Ageng Tirtayasa
151	Wilmar Maarisit	Universitas Kristen Indonesia Tomohon
152	Yatim Lailun Ni'mah	Institut Teknologi Sepuluh Nopember
153	yayuk astuti	Universitas diponegoro
154	Yehezkiel Steven Kurniawan	Universitas Gadjah Mada
155	Yuni K. Krisnandi	Universitas Indonesia
156	Yuniawan hidayat	Universitas sebelas maret
157	Philip Anggo Krisbiantoro	Academia Sinica Taipei Taiwan
158	Kristian Handoyo Sugiyarto	Universitas Negeri Yogyakarta

Dekan,

Prof. Dr.Eng. Kuwat Triyana, M.Si.



Dokumen ini telah ditandatangani secara elektronik menggunakan sertifikat elektronik yang diterbitkan oleh BSrE.

3. BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY (Q3 = 0,34)

Marlin UNIB <marlin@unib.ac.id>

Review Request - [Biointerface Res Appl Chem - Q3 Journal]

no-reply@jams.pub <no-reply@jams.pub> 26 Oktober 2021 pukul 11.41
Balas Ke: al.grumezescu@gmail.com
Kepada: Marlin Marlin <marlin@unib.ac.id>
Cc: BRIAC Editorial Office <al.grumezescu@gmail.com>

Dear Dr. Marlin Marlin,

We have received the following manuscript to be considered for publication in Biointerface Research in Applied Chemistry and kindly invite you to provide a review to evaluate its suitability for publication:

Type of manuscript: Article
Title: Metabolite profiling of Curcuma zanthorrhiza varieties grown in different regions using UHPLC-Q-Orbitrap-HRMS and chemometrics analysis

The abstract is available at the end of this message. Please click on the link below to access the manuscript and review report form, and inform us whether or not you will be able to provide a review.

https://jams.amgtranscend.org/user/review/review/1559/gzN6B8eQ

If you accept this invitation we would appreciate receiving your comments within 1 week. We would like to stress that we rely on the critical reviews of external experts to maintain the quality of BRIAC. Along with the authors, we would greatly value your contribution to the peer-review process.

If you are not able to review this manuscript, we kindly ask you to decline by clicking on the above link so that we can continue processing this submission. We would also appreciate any suggestions for alternative expert reviewers.

Please note that this peer-review request and the contents of the manuscript are highly confidential. You must not distribute the manuscript in part or whole to a third party, including other members of your research group, without explicit permission from the editorial office. You must also disclose if you have a conflict of interest with the content of the manuscript or the authors.

The BRIAC is one of AMG Transcend Association's open access journals. Our aim is to process manuscripts quickly and publish them shortly after peer-review.

Thank you very much for your consideration and we look forward to hearing from you.

Kind regards,

Alex

____
Alexandru Mihai GRUMEZESCU
http://grumezescu.com/
al.grumezescu@gmail.com
Editor in Chief
Biointerface Research in Applied Chemistry

Manuscript details:
Journal: Biointerface Research in Applied Chemistry
Type of manuscript: Article

Abstract: Curcuma zanthorrhiza, also known as java turmeric, is a plant that has long been used as a medicinal herb. The efficacy of C. zanthorrhiza is primarily determined by the bioactive composition, which is dependent on many variables, including where it is grown and the different varieties of java turmeric used. In this study, we determined the metabolite profile of C. zanthorrhiza 70% ethanol extract using UHPLC-Q-Orbitrap-HRMS coupled with chemometrics analysis to characterize the differences between C. zanthorrhiza varieties (namely Cursina-1, Cursina-2, and Cursina-3) grown in Bogor, Cianjur, and Sukabumi, West Java, Indonesia. An estimated total of 39 metabolites has been putatively identified. These metabolites were divided into amino acids, terpenoids, phenolics, diarylheptanoids, and other organic compounds groups. Chemometric results revealed significant differences in the geographical location metabolites profiles, which C. zanthorrhiza varieties had little effect. This study shows that UHPLC-Q-Orbitrap-HRMS-based metabolomics is efficient for profiling C. zanthorrhiza across various regions.

Marlin UNIB <marlin@unib.ac.id>

Review Request Accepted

no-reply@jams.pub <no-reply@jams.pub> 30 Oktober 2021 pukul 16.38
Balas Ke: al.grumezescu@gmail.com
Kepada: Marlin Marlin <marlin@unib.ac.id>

Dear Marlin Marlin,

Thank you very much for agreeing to review this manuscript:

Manuscript ID: briac-431
Type of manuscript: Article
Title: Metabolite profiling of Curcuma zanthorrhiza varieties grown in different regions using UHPLC-Q-Orbitrap-HRMS and chemometrics analysis

The review report form can be found here:
https://jams.amgtranscend.org/user/review/review/1559/gzN6B8eQ

We look forward to receiving your valuable comments.

Kind regards,

Reviewer Report

Biointerface Research in Applied Chemistry Journal

Comment of Manuscript

Title : Metabolite profiling of Curcuma zanthorrhiza varieties grown in different regions using UHPLC-Q-OrbitrapHRMS and chemometrics analysis

	We recommend that you use a larger number of plant materials and come from diverse areas.
	Sampling should be sourced from areas that have different geographical and environmental conditions, not only in West Java.
	In the method, it is necessary to explain in detail the environmental conditions of the study (soil pH, temperature, humidity, nutrient status), explain the differences in environmental conditions of the two experimental fields. What factors determined that these two locations were chosen as research sites?
	The sample pretreatment and metabolite extraction steps from raw leaves into ready-injected samples should be described in the methodology.
	The sample set information, including the numbers of biological and technical replication, and the quality control samples should be provided in methods to make sure that the data set is statistically enough.
	When performing metabolomics data analysis, there are huge spectra data collected from the analysis. Filtering and clustering spectra data must be done before further analysis. These steps were crucial to perform prior to metabolite identification. However, these steps were not described in the methodology. Please include these steps in the method.
	However, the results were not delivered in a clear state, so it is difficult to follow and understand it. For example, the author describes a particular metabolite specifically present in different geographical, but the author does not describe from which treatments the metabolite is produced. It is necessary to explain the specific metabolites of *C. zanthorrhiza* which were found in this study as a result of differences in varieties and experimental fields.

	In addition, significant data cited in the discussion is not present in the manuscript. It is vital that the author must provide metabolite information to understand the hypothesis raised in the discussion. It may also reconsider to analyze metabolites from the whole plant organs to get a big view of metabolites responsible in *C. zanthorrhiza*
	References suggested in Literature Review Section should be included
	Other minor comments are the redundancy of word usage of metabolite and compound is applied together and all Figures were not supported in enough resolution, so it is difficult to understand the content. In addition, there are different font style used in the manuscript and some grammatical errors are detected.

Recommendation : Requires Moderate Revision

Signature of the Reviewer

November 7. 2021

Marlin UNIB <marlin@unib.ac.id>

Review Received - Thanks

no-reply@jams.pub <no-reply@jams.pub> 8 November 2021 pukul 22.16
Balas Ke: al.grumezescu@gmail.com
Kepada: Marlin Marlin <marlin@unib.ac.id>
Cc: al.grumezescu@gmail.com

Dear Marlin Marlin,

A short note to thank you very much for your review of the following manuscript:

Manuscript ID: briac-431
Type of manuscript: Article
Title: Metabolite profiling of Curcuma zanthorrhiza varieties grown in different regions using UHPLC-Q-Orbitrap-HRMS and chemometrics analysis

Your careful review is valuable to us in making a decision regarding this paper, and in contributing generally to the quality of work published in Biointerface Research in Applied Chemistry.
Kind regards,

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