BUKTI KORESPONDENSI

ARTIKEL JURNAL INTERNASIONAL BEREPUTASI (TERINDEKS PADA DATABASE INTERNASIONAL BEREPUTASI DAN BERDAMPAK FAKTOR)

JudulArtikel : Host range studies of Fusarium oxysporum, causal agent of seedling wilt Disease of Acacia mangium

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Penulis : Soleha, **Ahmad Muslim*(sebagai Corresponding Author)**, Suwandi, Sabaruddin Kadir, Rahmat Pratama

No.	Perihal	Tanggal
I.	**SUBMISSION PROCESS** 1. Bukti konfirmasi Submission Process	22 September 2021
II.	**REVIEW PROCESS** 1. Bukti konfirmasi review pertama dari reviewer	17 November 2021
	2. Bukti konfirmasi revision of manuscript pertama, respon kepada reviewer dan perbaikan revisi artikel yang di resubmit	26 November 2021
	3. Bukti konfirmasi review kedua dari reviewer	29 November 2021
	4. Bukti konfirmasi revision of manuscript kedua, respon kepada reviewer dan perbaikan revisi artikel yang di resubmit	02 Desember 2021
	5. Bukti konfirmasi review ketiga dari reviewer	04 Desember 2021
	6. Bukti konfirmasi revision of manuscript ketiga, respon kepada reviewer dan perbaikan revisi artikel yang di resubmit	06 Desember 2021
III.	**PROOFREAD AND PUBLISHING PROCESS** 1. Bukti konfirmasi pembayaran jurnal	08 Desember 2021
	2. Bukti konfirmasi accepted letter	16 Desember 2021
	3. Bukti konfirmasi artikel untuk di published online	16 Desember 2021

SUBMISSION PROCESS

1. Bukti konfirmasi Submission Process

a.muslimunsri<a_muslim@unsri.ac.id>

[biodiv]Submission Acknow ledgement

1 message

Ahmad Dwi Setyawan <smujo.id@gmail.com> Wed, Sep 22, 2021 at 2:10 PM
To: Ahmad Muslim <a_muslim@unsri.ac.id>

AhmadMuslim:

Thank you for submitting the manuscript, "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd." to Biodiversitas Journal of Biological Diversity. With the online journal managementsystemthatweareusing,youwillbeabletotrackitsprogressthroughtheeditorialprocessbylogging in to the journal web site:

Submission URL: https://smujo.id/biodiv/authorDashboard/submission/9450
Username: amuslim

If you have any questions, please contact me. Thank you for considering this journal as a venue for your work.

Ahmad Dwi Setyawan

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1. Bukti konfirmasi review pertama dari reviewer

a.muslimunsri<a_muslim@unsri.ac.id>

[biodiv]EditorDecision

2 messages

Smujo Editors <smujo.id@gmail.com> Wed, Nov 17, 2021 at 1:12 PM
To: Soleha Soleha <soleha057@gmail.com>, Ahmad Muslim <a_muslim@unsri.ac.id>, Suwandi Suwandi <Suwandi@fp.unsri.ac.id>,SabaruddinKadir<sabar@pps.unsri.ac.id>,RahmatPratama <rahmatpratama@pps.unsri.ac.id>

SolehaSoleha,AhmadMuslim,SuwandiSuwandi,SabaruddinKadir,RahmatPratama:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd.".

Ourdecisionis:RevisionsRequired

- - - - - - - - - - - - -

ReviewerA:

Comments:

1.Writereferencesaccordingtojournalrules.

* Akond MA, Jahan MN, Sultana N, Rahman F. 2016. Effect of temperature, pH and NaCl on the isolates of actinomycetes from straw and compost samples from Savar, Dhaka, Bangladesh.Am J Microbiol and Immunol 1: 10-15.

* Barbosa J, Albano H, Silva B, Almeida MH, Nogueira T, Teixeira P. 2021. Characterization of a *Lactiplantibacillus plantarum* R23 isolated from arugula by whole-genome sequencing and its bacteriocin production ability. Int J Environ Res Public Health 18: 5515.

1. Somelinesareveryconfusingsopleasecorrectthem.
2. Youhaven’twrittenanyreferenceinthetextso,pleaseaddreferencesintext.
3. Pleasecorrectfigure8.Thereshouldbepointnotcomma.
4. Table1.didnotshowdiseaseincidencesopleasecheckit.
5. Pleasereadpapercarefully.

Recommendation:SeeComments

- - - - - - - - - - - - -

2. Bukti konfirmasi revision of manuscript pertama, respon kepada reviewer dan perbaikan revisi artikel yang di resubmit

BiodiversitasJournalofBiologicalDiversity

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811K

a. muslim unsri <a_muslim@unsri.ac.id> Fri, Nov 26, 2021 at 8:56 AM
To: Smujo Editors <smujo.id@gmail.com>

November26,2021

13/01/2214.36 SriwijayaUniversityMail-[biodiv]EditorDecision

DearEditorinChief
BiodiversitasJournalofBiologicalDiversity

Thank you very much for your email regarding reviewer's recommendation, suggestion, and revision of our manuscript. We would like to thank and appreciate for all reviewers'suggestions and corrections.

We have made corrections and some modification according to Reviewer's revisions. Here, we enclose revisedmanuscriptwithtrackedchangesandhighlightofthemanuscriptentitled"HostRange Studiesof*Fusariumoxysporum*,CausalagentofSeedlingWiltDiseaseof*Acaciamangium*"bySoleha Soleha,AhmadMuslim,SuwandiSuwandi,SabaruddinKadir,RahmatPratama.

In this opportunity, we would like to re-submit our revised manuscript for publication in Biodiversitas Journal of Biological Diversity.

Belowisasummaryofourchangesmadeinresponsetothereviewer'scomments.

1. ***Reviewer'scomment:***Writereferencesaccordingtojournalrules.
* AkondMA,JahanMN,SultanaN,RahmanF.2016.Effectoftemperature,pHandNaClontheisolates of actinomycetes from straw and compost samples from Savar, Dhaka, Bangladesh.Am J Microbiol and Immunol 1: 10-15.

* BarbosaJ,AlbanoH,SilvaB,AlmeidaMH,NogueiraT,TeixeiraP.2021.Characterizationof Lactiplantibacillus plantarumR23isolatedfromarugulabywhole-genomesequencingandits bacteriocin production ability. Int J Environ Res Public Health 18: 5515.

Ourresponse: Actually, we have written the refence according to journal rules. We check andrecheck again, there are a few mistakes as reviewer's comments. We have revised and changed our manuscript.

2. ***Reviewer'scomment:***Somelinesareveryconfusingsopleasecorrectthem

Ourresponse: Wechangedthesentencesasfollows.

Reviewer'scomment:Introduction,line20:Writetheauthenticityofplant.
Ourresponse: Theauthenticityofplanthasbeenaddedinmanuscript.(p:1,line:19ofrevisedmanuscript)

Reviewer'scomment:Materialsandmethod,line44-45: Rewritethisline.Orremovethisline" utilised;theywerechosenbecauseapreviousstudy(haddescribedthemasthemostpathogenicto*A. mangium"*
Our response: We are appreciating for this comment, we agree and remove the sentence. (p: 2, line: 47 of revised manuscript)

Reviewer'scomment:Materialsandmethod,line64:Writetheauthorname,rewriteit"themethod designed by and modified using a disease index"
Our response: We have rewritten and changed the manuscript with "the method designed by Muslimet al. (2003a) and modified using a disease index "(p: 2, line: 66)

Reviewer's comment: Materials and method, line 76:Correct the name of media "Peptone PCNBagarmedia".
Ourresponse: Thenameofmediais"PeptonePCNBagarMedia(PPA/NashSnyderMedium)" according toLeslieandSummerell(2006).(p:2,line:77-78ofrevised manuscript)

Reviewer'scomment:Materials and method, line 79:I can't understand the meaning of this line what do you want to record "recorded based on the disease severity". Rewrite it.

Ourresponse: Thechangedhasbeenmadetobe"Thenumberofcolony-formingunits(CFU)of *F. oxysporum* was calculated on the basis of fresh weight per gram of sample and grouped according to the diseases severity level. (p: 2, line: 79-81 of revised manuscript)

Reviewer'scomment:Results,figure7.line113:Writetheadvancesymptoms.
Ourresponse: We have changed description of Figure 7 and modified the picture (p: 5, line 131-132 of revised manuscript)

Reviewer'scomment:Results,line119:This16%diseaseincidencewasnotmentionedintable 1.
Ourresponse: Data of diseases incidence is not shown in the manuscript. We remove the sentence"and 16% incidence (p: 5, line: 138 of revised manuscript)

Reviewer's comment :Result, line 123 "Although the three isolates had different tef1 geneticsequencing, they had similar virulence patterns".I removed this line because you did not mentionthe sequencing of isolates.
Ourresponse: We agree and remove the sentence "Although the three isolates had different tef1 genetic sequencing, they had similar virulence patterns". (p: 5, line 142 of revised manuscript)

Reviewer'scomment:Results,line140:Ican'tunderstandit.Rewriteit.Whatsamples?
Ourresponse: We change and rewrite the sentence from "Thissame pattern alsooccurred inDI 2 and3, while no sample was recorded in*A.auriculiformis*"tobe"This same pattern also occurred in DI 2 and 3, while no sample was recorded in*A. Auriculiformis* for DI 2 and 3". (p: 5, line: 137-138 ofrevised manuscript).

Reviewer'scomment:Results,line140-141:Ican'tunderstandit.Rewrite it.
Ourresponse: We change and rewrite the sentence to be "In DI 1, the highest population wasrecorded in *F. moluccana* and*L. leucocephala*, while*A. crassicarpa* and*A. auriculiformis* had no sample forDI 1". (p: 5, line: 138-139 of revised manuscript).

Reviewer's comment :Discussion, line 179-180:Which recent study and from where this isreported. Correct it.
Our response: The sentence has been corrected to be " A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen*F. oxysporum* attackingcommercial nurseriesof*A.mangium*inSouthSumatra(Solehaetal.2021)(p:8,line180-181ofrevisedmanuscript).

Reviewer'scomment:Discussion,line190-191:Writethissentenceproperly.
Ourresponse: We change the sentences to be "Asimilar incident was reported by Pastrana et al. (2017) in which*F. oxysporum* from blackberry also caused sudden death in strawberries. Another studyalso revealed that*F. oxysporum* from cactus causes root and stem rot also causes diseases onEuphorbia (Bertetti et al. 2017)". (p: 8, line 192-193 of revised manuscript).

Reviewer's comment :Discussion, line 207-208: Please rewrite this line. Is this line can be written like this "This pattern was common where the population of the pathogen was also higher with the disease scores".
Ourresponse: We agree and are grateful for this suggestion, the sentence has been changed to be "This pattern was common where the population of the pathogen was also higher with the disease scores". (p:8, line 204-205 of revised manuscript).

Reviewer'scomment:Discussion,line209-210:Rewritethisline.Ican'tunderstandthemeaning"Inthe second

pattern, the population was moderate in a moderate susceptible host (*L. leucocephala*)".
Our response: The sentence has been re-written to be "The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate diseases score". (p: 8, line: 207-209 of revised manuscript).

Reviewer's comment :Discussion, line: 213-214: Rewrite this line. I can't understand the meaning: "A special pattern occurred on*A. pauciflorum* that could have been caused by a moderatepathogen infection, but the pathogen population was low".
Ourresponse: The sentence has been re-written to be "A special pattern occurred on*A.pauciflorum*that *F.oxysporum* caused a moderate infection, but the pathogen population was low". (p: 8, line: 212-213 of revised manuscript).

Reviewer'scomment:Discussion,line226:Checkitagain:"race 0".
Ourresponse: Theword(race0)iscorrect(Jiménez-Fernándezetal.(2013)).

3. ***Reviewer's comment:*** You haven't written any reference in the text so, please add references in text.
Ourresponse: WedoapologizeforthemistakeofreferencesthatwewriteintheMendeleyformat automatically. Therefore, it can't be read by reviewer. All of the reference has been added in manuscript. (p: 1-10, line: 19-314 of revised manuscript).

4. ***Reviewer'scomment:***Pleasecorrectfigure8.Thereshouldbepointnotcomma.

Ourresponse: Wehaverevisedfigure8bychangingcommatopoint.

Reviewer's comment :Results, line 156:Y=20.327and8.5833thereshouldbepointandcomma, please correct it all the rest of the figures 8.
Ourresponse: Thechangedhasbeenmadeonfigure8.inmanuscript.(p:6,line;156-157ofrevised manuscript)

5. ***Reviewer'scomment:***Table1.didnotshowdiseaseincidencesopleasecheck it.

Reviewer's comment: Results,line 118, description of Table 1 :Here you write severity and in textyou wrote incidence. Check it again.
Our response: We are appreciating for this comment, we record diseases severity and modified the sentence for description of Table 1, from "Pathogenicity and disease severity of *Fusarium oxysporum* isolated from*Acaciamangium* "to "Disease severity and host responses to *Fusariumoxysporum* isolated from *Acacia mangium*. (p: 5, line 120 of revised manuscript).

6. ***Reviewer'scomment:***Pleasereadpapercarefully.Recommendation:SeeComments

Our response: We have read our paper carefully and we changed all the words/sentences as reviewer's comment, suggestion and revision.
-
We feel that these changes have adequately addressed the comments and suggestions of the reviewers,and we look forward to publication in the Biodiversitas Journal of Biological Diversity.

Pleasefeelfreetocontactmeifyouneedanyadditionalinformationorclarification. Thank you very much for your excellent cooperation

Sincerely,
AhmadMuslim
Senior lecturer
FacultyofAgriculture,SriwijayaUniversity
Jl.Palembang-PrabumulihKm.32,Indralaya,Palembang,Indonesia E-mail: a_muslim@unsri.ac.id

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Review1

Host range studies of *Fusariumoxysporum*, ~~the~~ causal agent of seedling wilt disease of *Acaciamangium*

Abstract. *Fusariumoxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acaciamangium* in South Sumatra. It has been reported to have a high level of host specification at the genus or family level. This study aimed to investigate the host range of *F. oxysporum* as a nurserywilt pathogen on in *A. mangium* and several forests and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef*1-α) sequences were tested for pathogenicity on different fabaceae family plants from the Fabaceae family and the growth of their population was also observed. The results showed that it can all the three isolates were able to infect all the tested plants with different reaction sofwilt disease. The *Acaciacrassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendronpauciflorum*, *Leucaenaleucocephala*, and *Parkiaspeciosa* were moderately vulnerable and *Acaciauriculiformis* was moderately resisted resistant *by Acacia~~auriculiformis~~*. ~~The pathogen~~ population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while that of in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, the *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the Fabaceae family.

Keyword: *Acaciamangium*, Fabaceaefabaceae, *Fusariumoxysporum*, host range, seedling wilt

INTRODUCTION

Acaciamangium(. . .) is a species of plant that originated in several regions in of Indonesia, Papua New Guinea, and Australia, and which, ~~for a few decades~~, has also been found for a few decades introduced to in the ~~humid~~ tropical lowlands of Asia, South America, and Africa (. . . .). It ~~was is~~ planted on a large scale for industrial purposes and forest restoration in the tropics. Since this plant species is known for its fast growth and high adaptability to various environmental conditions, it ~~is~~ is widely used in for ~~agro~~ forestry, forestry, and ~~for~~ restoration of degraded land.

Fusariumoxysporum is an important pathogenic fungus that causes wilt disease in different plants all over the world. . . . . reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant. Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds). This fungus is also able to attack various plant habits such as trees, herbaceous plants, and vines. Several types of forest plants that have reported lybeen attacked by *F. oxysporum* this fungi are *Pi~~nusmassoniana~~*, *Tectonagrandis*, *Pseudotsugamenziesii*, *Acaciamangium* and others (. . . . .). . . . . .

Since *F. oxysporum* has a high level of host specificity, it is classified as a formaespecies . . . According to . . . . . , more than 100 formaespecies and race shave been identified and are wide spread in the world.

Besides *A. mangium*, which is the main ~~commodity~~ plant of industrial forestry in Indonesia, other plants, such as, *Acaciacrassicarpa*, *Acaciauriculiformis*, *Parkiaspeciosa*, *Archidendronpauciflorum*, *Falcatariamoluccana*, and *Leucaenaleucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can become the main or alternative hosts for *F. oxysporum*, the causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nurserywilt pathogen ~~on~~ in *A. mangium* and ~~s~~ several industrial and local forest plants in Indonesia.

MATERIALSANDMETHOD

Fungalisolates

Three isolates of *F. oxysporum* (AF01, BF05, and DF11), ~~which~~ were selected, which were differentiated according to their *tef*1-α sequence, were utilised; they were chosen because a previous study (had described the as the most pathogenic to *A. mangium* (Figure1). ~~Pathogenisolates~~ Isolates were cultured in on a PDBliquidmedium (potato dextrose broth) and incubated at 26-28°C on a shaker (150rpm) for about five days. The the myceliasuspension ~~produced~~ was

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filteredusingtwolayersofsterilegauzetoseparatetheconidiaandhyphae.Theconidialconcentrationwasdetermined usinga hemocytometer and then~~diluted~~adjustedtoa concentrationof10^6 ml^{-1} forthe pathogenicitytest.

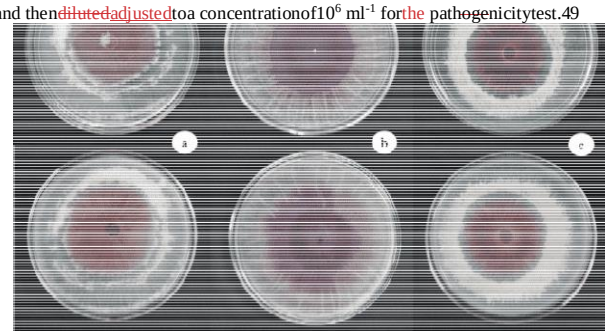

Figure1.*F.oxysporum*isolatesonPDAmedium.(a)AF01,(b)BF05,and(c)DF11.Firstline:topsurfaceFrontview;secondline: bottomsurfacereverseview.

Plantmaterial

Theplantsusedweremembersofthe~~Fabaceae~~fabaceae~~(legumes)~~family,namely,*A.crassicarpa*,*A.auriculiformis*, *F.moluccana*,*A.pauciflorum*,*P.speciosa*,and*L.leucocephala*,whichwereonemonthold.Theseedlingswereobtained fromtheForestCropsResearchInstitute,SouthSumatra.,Seedlingsweretransferredinamixedmediumwithcocopeat (1:1)usingaplasticpotof10cmthathad~~a~~diameterand~~aheightof~~10cmheight,and~~was~~thenplacedinashadehouse.

Pathogenicitytest

Apathogenicitytestwascarriedoutusingtherootdipmethod,~~where~~inwhichtherootswerewashedunderrunning waterandthenimmersedin250mlofconidiasuspension(10^6conidiaml^{-1})for15minutes.Thecontrolplantswere immersedinsteriledistilledwater,andtheseedlingsweretransplantedintoplasticpotsandplacedunderahouseshade. Eachisolatewasinoculatedon25plantswithfivereplicates(fiveplantsper-replicate).Then,diseaseseveritywas calculatedusing themethoddesignedbyandmodifiedusingadiseaseindex (DI)0-4,where0=nodisease/healthy seedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severewilt,and4=deadseedling.Furthermore, plantresponseswereregroupedas,R=resistant(D.I.=0),MR=moderatelyresistant/tolerance(DI=<1),MS=moderately susceptible(DI=1.0-2.0),S=susceptible(DI=2.1-3.0)andHS=highlysusceptible(DI=3.1-4.0).Thedevelopmentof ~~the~~diseasewasobserved~~for~~1–21daysafterinoculation.

***Fusariumoxysporum*population**

Thepopulationof*F.oxysporum*intherootswascalculatedattheendoftheexperimentusingthemethodof(Muslim etal.2003b;Lietal.2008;Horinouchietal.2011)withmodificationstothesurface~~sterilisation~~sterilizationofthe samples.Thentheplantsweregroupedaccordingtoseverity(diseasescore)andwashedseparatelyunderrunningwaterto remove~~any~~soilresidues~~adhering~~~~to~~~~the~~~~roots~~.Afterthat,allplantsineachscoreweresurfacesterilisedusing1%sodium hypochloritefor15minutes,thenrinsedthreetimeswithdistilledwater.Thesamplesandwater(1:100w/v)were homogenisedusing~~a~~blenderat~~aspeedof~~8000rpmforten10min~~utes~~.Thentheywerefilteredusingtwolayersofsterile gauzeanddiluted10to1000times.Thesuspensionwasspreadon PeptonePCNBagarmedia intriplicate(fivePetri dishesperreplication)andincubatedinthedar~~k~~~~for~~sevendaysatroomtemperature.Thenumberofcolony-formingunits (CFU)of*F.oxysporum*wascalculatedonthebasisoffreshweightpergramofsample~~pergrammeofsample's~~~~fresh~~ ~~weight~~andrecordedbasedonthediseaseseverity.

RESULTSANDDISCUSSION

Pathogenicitytest

~~ThreeisolatesofF.oxysporumweretestedonsixtypesofforestplantsandthe~~The resultsshowedthatallthesix forestplantstestedhadasimilarreactiontothepathogen.Sevendaysafterinoculation,alltheplantsshowedtypical symptomsof*F.oxysporum*infection,i.e.,yellowingofthe~~old~~~~est~~leavesclosesttothestembase,whichgraduallyprogress

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to~~those~~younger~~andthe~~shoots,severewilting,drying,~~and~~fallingofleaves,and~~lastly~~eventuallyplantdie,death.
Anothersymptomthatappearedwas~~the~~suddenwiltinganddeathof~~the~~plantwithout~~thechange~~changing~~of~~theleaves' leafcolour,~~; meanwhile, the~~ whilecontrol plants didnot showanysymptoms (Figure 2, 3,4,5,6 &7).

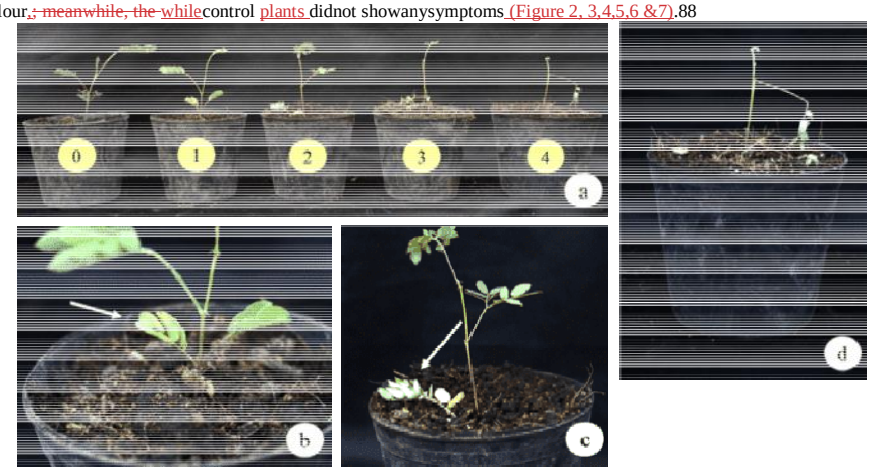

Figure2. DiseaseindexofA*cacia crassicarpa*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initialsymptoms: yellowingfromoldestleaves;(c)advancedsymptoms:fallingleaves;(d)deadplant.

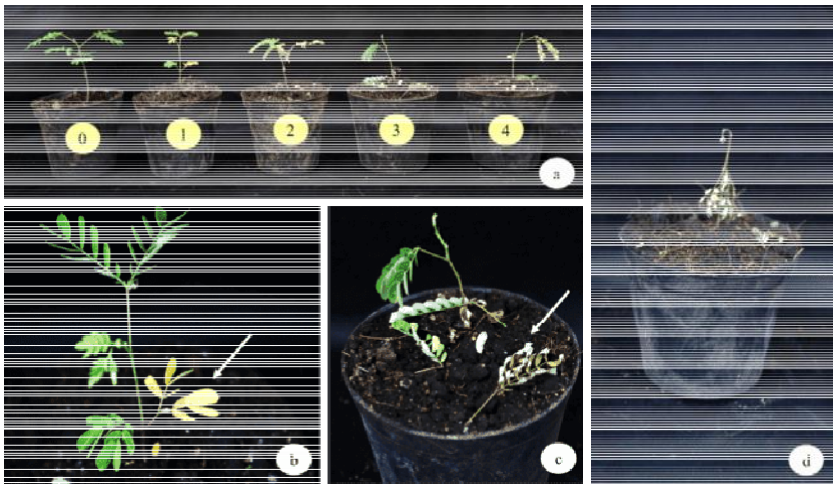

Figure3. Diseaseindex on*Falcatariamoluccana*(a)from left: healthy plantto 100%wiltedleaves(score0−4); (b) initialsymptoms: yellowingfromoldestleaves;(c)advancedsymptoms:curved,dry,andfallingleaves;(d)deadplant.

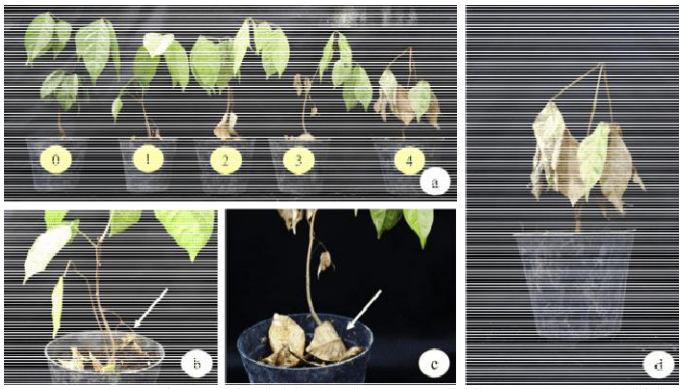

Figure4. Disease index on *Archidendron pauciflorum*, (a) from left: healthy plant to 100% wilted leaves (score 0−4), (b) initial symptoms: yellowing and dry from oldest leaves, (c) advanced symptoms: falling leaves, (d) dead plant.

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Figure5. Diseases index on *Leucaena leucocephala*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward.

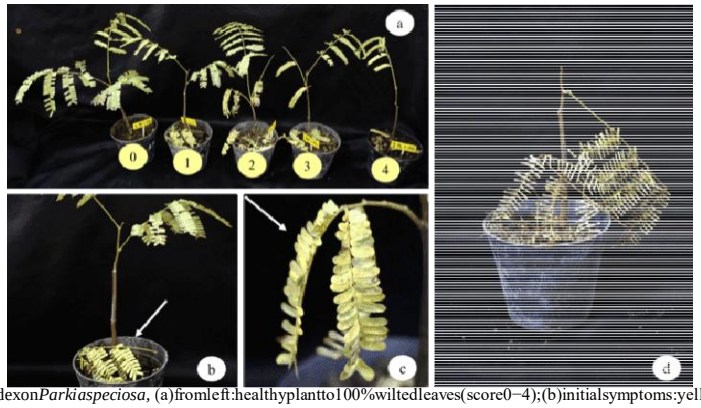

Figure6. Diseases index on *Parkia speciosa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant.

Figure 7. Diseasesindexon*Acaciaauriculiformis*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(bandc)advanced symptoms: ……..

Diseaseseverity~~causedbytheinoculated *F.oxysporum*isolates~~wassignificantlyhigherthan~~the~~controls. *A.crassicarpa*and *F.moluccana*weremostseverelyaffectedwithanaveragescoreof4.00and3.44,respectivelyandtheincidence of wilting was 100%. On the other hand, *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were ~~attacked~~ showed~~with~~moderate disease severity ~~(scores~~ i.e. 1.96, 1.68, and 1.80, respectively), ~~and~~ whereas *A. auriculiformis* had the lowest(0.36)diseaseseverityand~~1~~(16%)incidence~~(16%and0.36)respectively)~~(Table1).Basedon~~thediseasescoreonplant~~response,~~~~thesearethethreegroupsofresponsesbythehosts~~Basedonthediseasescore,hostplantswereclassifiedintothreegroups:i)highlysusceptible(*A.crassicarpa*and *F.moluccana*),ii)moderatelysusceptible(*A.pauciflorum P.speciosa*, and *L. leucocephala*), and iii) moderate resistance/tolerance (*A. auriculiformis*) (Table 1).

~~Althoughthethreeisolateshaddifferent*tef1*-geneticsequencing,theyhadsimilarvirulencepatterns~~

Therefore,Result exhibited that there was no significant difference between the disease severity in the same host that ~~was~~ had beeninoculated with different isolates.

Table1. Pathogenicityanddiseaseseverityof*Fusariumoxysporum*isolatedfrom*Acaciamangium*

Plantspecies	Isolates[a)]					
	AF01[b)]Response		[c)]BF05Response		DF11Response	
Acaciacrassicarpa	4.00a	HS[c)]	3.48 a	HS	3.96a	HS
Falcatariamoluccana	3.44ab	HS	3.04 a	HS	2.80ab	S
Archidendronpauciflorum	1.96bc	MS	1.88 b	MS	1.40cd	MS
Leucaenaleucocephala	1.52c	MS	1.56 b	MS	1.68bc	MS
Parkiaspeciosa	1.80c	MS	1.04 bc	MS	2.16bc	S
Acaciaauriculiformis	0.36d	MR	0.40 c	MR	0.60d	MR

Valuesfollowedbythesameletterineachrowarenotsignificant.

[a] DI0-4,where0=nodisease/healthyseedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severewilt, and4=deadseedling.

[b)]*F.oxysporum*isolates.

[c)] Hostresponseweregroupedas:R=resistant(D.I.=0);MR=moderatelyresistant/tolerance(D.I.=<1);MS=moderatelysusceptible (D.I.=1.0-2.0);S=susceptible(D.I.=2.1-3.0);HS=highlysusceptible(D.I.=3.1-4.0)(Bertettietal.,2018)

***~~F.~~Fusarium*oxysporum*population**

Thetotalpopulationof *F.oxysporum*ontherootswasdeterminedbycalculatingtheCFUforeachcategoryofdamage. The results showed that at a score of 4, ~~the three isolates which were tested on~~ *A. crassicarpa* and *F. moluccana*showed a significantly higher population (82.00–105.10 × 10^4 CFU g^{-1} fresh weight) than ~~the~~other plants. The lowestpopulationwas recorded~~on~~in *P. speciosa* and *A. pauciflorum* (3.57–12.27 × 10^4 CFU g^{-1} fresh weight). This same patternalso occurred in DI 2and 3, whileno samplewasrecordedin *A.auriculiformis*. In DI 1, thehighestpopulation was~~discovered~~recordedin *F.moluccana*and *L.leucocephala*,while *A.crassicarpa*and *A.auriculiformis*h adnosample.

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~~Meanwhile,in~~In~~asymptomatic~~control plants (DI=0), the population was significantly higher in *L. leucocephala* and *A. auriculiformis* and no sample was ~~discovered~~ noted in *A. crassicarpa* and *F. moluccana* (Table2 ~~–~~ & Table3).

The regression analysis results showed that all plants except *P. speciosa* had a linear relationship pattern between the increase in the ~~di~~seases core and the pop~~ula~~tion. The pathogenic population on *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in diseases scores, as indicated by the magnitude of the regression grad~~ien~~t coefficient (m=20.3–21.3). However, the ~~mo~~derate increase was ~~moderate~~observed in *L. leucocephala* (m=11,.2) (m=11.2) and very slow in *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* (m=2,.2–4,.8) (Figure.8).

Table3 ~~shows~~showed that ~~although the~~ isolates were different in *tef1-α*, but the population and DI patterns were similar for each test plant. The correlation between the population of pathogens (g^{-1} fresh weight) and the level of DI ~~are~~ were described as follows: i) high pathogen populations with high DI (*A. crassicarpa* and *F. moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population with moderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).

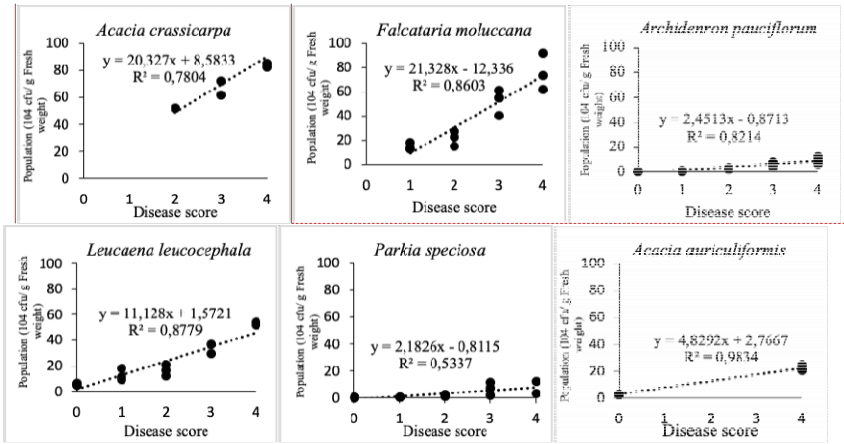

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Figure8. Regression analyses analysis of diseases core rate and *F. oxysporum* population

Table2. *Fusarium oxysporum* populations on root in each disease index

Plant species	Population of *Fusarium oxysporum* (×10^4 CFU/g fresh weight)[a]					Average[c]
	0[b]	1	2	3	4	
AF01[d]						
Acacia crassicarpa	n.s	n.s	n.s	n.s	85.13a[e]	85.13
Falcatariamoluccana	n.s	17.77a	22.77a	60.98a	91.87a	76.50
Archidendron pauciflorum	0.45 b	1.10b	3.22b	8.15b	12.53cd	5.06
Leucaena leucocephala	6.17a	18.10a	20.93a	n.s	51.67b	22.13
Parkia speciosa	0.32 b	0.45b	2.58b	7.27b	3.57d	2.16
Acacia auriculiformis	2.92a	n.s	n.s	n.s	24.53c	4.65
BF05						
Acacia crassicarpa	n.s	n.s	51.80a	72.08a	105.10a	92.61
Falcatariamoluccana	n.s	13.22a	15.32b	40.33b	61.67b	43.85
Archidendron pauciflorum	0.47c	0.63b	1.73c	6.88c	9.90d	3.60
Leucaena leucocephala	4.67a	9.02a	12.32b	29.32b	n.s	11.16
Parkia speciosa	0.48c	0.57b	1.27c	2.33d	n.s	0.87
Acacia auriculiformis	2.55b	n.s	n.s	n.s	20.43c	3.98
DF11						
Acacia crassicarpa	n.s	n.s	n.s	61.92a	82.00a	81.20
Falcatariamoluccana	n.s	12.50a	27.47a	54.93a	73.00a	47.93
Archidendron pauciflorum	0.35c	0.35b	3.37c	4.42c	6.92e	2.19
Leucaena leucocephala	5.58a	11.17a	16.53b	36.63b	54.27b	19.69
Parkia speciosa	0.25c	0.48b	1.58c	11.97d	12.27d	5.79
Acacia auriculiformis	2.83b	n.s	n.s	n.s	21.28c	5.05

n.s: No sample, cfu: colony-forming unit

[a] *F. oxysporum* populations ~~were~~ calculated at the end of the experiment (21 days after inoculation).

[b] DI 0-4; 0=no disease/healthy seedling; 1=yellow leaves; 2=yellow leaves and slightly wilted; 3=severe wilt; and 4=dead seedling.

[c] Average of *F. oxysporum* population (cfu/g fresh weight) =($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, ~~dan~~ and 4 ~~respectively~~: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score 4; N = total number of plants.

[d] *F. oxysporum* isolates

[e] Values followed by the same letter in each row are not significant.

Table3. *Fusariumoxysporum*populationaverageanddiseasesindexofplant

Plantspecies	Populationaverage(×10^4CFU/gfresh weight)[a]			Diseaseindex[b]		
	AF01[c]	BF05	DF11	AF01	BF05	DF11
Acaciacrassicarpa	85.13	92.61	81.20	4.00	3.48	3.96
Falcatariamoluccana	76.50	43.85	47.93	3.44	3.04	2.80
Archidendronpauciflorum	5.06	3.60	2.19	1.96	1.88	1.40
Leucaenaleucocephala	22.13	11.16	19.69	1.52	1.56	1.68
Parkiaspeciosa	2.16	0.87	5.79	1.80	1.04	2.16
Acaciaauriculiformis	4.65	3.98	5.05	0.36	0.40	0.60

[a]Average of*F. oxysporum*population(cfu/gfreshweight)= ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N;whereP_0,P_1,P_2,P_3,andP_4=population ofpathogeninscore0,1,2,3,~~dan~~and4~~respectively~~:A=numberofplantsonscore0;B=numberofplantsonscore1;C=numberof plantsonscore2;D=numberofplantsonscore3;E=numberofplantson score;N=totalnumberof plants.

[b]DI0-4;0=nodisease/healthyseedling;1=yellowleaves;2=yellowleavesandslightlywilted;3=severewilt;and4= deadseedling.

[c]*F.oxysporum*isolates.

Discussion

recentstudyreported anextraordinaryincidenceofseedlingwiltdiseasecausedby~~the~~fungalpathogen*F. oxysporum*attacking commercial nurseries of *A. mangium*-. Therefore, the investigation of a new host of the pathogen is animportantstepintheplantprotectionstrategyforsoil-bornediseases.Hostrangetestsalsoprovideinformation~~on~~about~~the~~plant species that have the potential to become alternative hostsormain hosts for the pathogen.

Theresultsindicatedthat~~the~~*F.oxysporum*~~pathogen~~,whichcausesvascularwiltin~~the~~*A.mangium*nursery,canalsoinfect~~Fabaceae~~fabaceaeplantswithvarioushostresponses.~~,asconfirmedbytheKochPostulatetest~~.*A.crassicarpa*and *F.moluccana*~~showeda~~wereshighlysusceptible~~reaction~~,while *A.pauciflorum, L.leucocephala*,and *P.speciosa*weremoderatelyvulnerable,and *A. auriculiformis*wasmoderatelyresistant.Pathogen~~infection~~caused wiltingsymptomsinalltestplantspecieswith~~by~~DIof4.00.Although ~~the~~DIwaslower(0.36)~~for~~in*A.auriculiformis*,butit~~still~~hadthepotentialto~~cause~~damageplants.~~damageif~~*F.oxysporum*~~managed~~ableto infectplantsevenwithalowDI,causingthedeathofcultivars.Moreover,whena plantisgrown~~on~~incontaminatedsoil,thereisahigh~~exposure~~~~occurs,whichleadsto~~riskofdamagetocrops.Asimilarincidentwasreportedby…….,inwhich *F.oxysporum*fromblackberryalsocausedsuddendeath in strawberries; therefore, it becomes a threat when planted on adjacent land. Another study also~~discovered reve~~……nat*F.oxysporum*fromcactuscausesrootandstemrotin~~euphorbia~~Euphorbiawhen~~planted~~andcultivatedonthesame land .~~Furthermore,*P.speciosa*,whichshowedwiltingofmorethan50%,hadalowseverityscore(asinthecaseof *A.auriculiformis*),whichmeanslowDIshouldbenotedalso~~.

Theresultsrevealedthatseveraltypesofplants~~that weretestedfrom~~belongingtothe~~Fabaceae~~fabaceaefamilyhadgreat potential to become an alternative hosts and even ~~the~~ main host for *F. oxysporum* when planted in the same field. ~~The~~Widespreadofthispathogen~~toawiderarea~~mayallowsinteractionswithnewplants~~inthefuture~~.Moreover,theplantingof new species affected the occurrence of new outbreaks because the pathogenic strains ~~had~~ adapted to the soil and had~~become~~became virulent.Furthermore,nurseryactivitiesthatusecontaminatedsoilrepeatedlyalsotriggeredthepathogens' proliferation and adaptation to other plants.

Thepathogenpopulationsin*A.crassicarpa*and*F.moluccana*grewveryrapidlywithincreasingdiseasescores,whilein *L. leucocephala* grew moderately, and *A. pauciflorum, P. speciose,* and *A. auriculiformis* grew slowly.~~The relationshipbetween the pathogens (g^{-1} fresh weight) and the DI level is as follows: i) high pathogen populations and high DI (*A.crassicarpa*and *F.moluccana*),ii)moderatepopulationandmoderateDI (*L.leucocephala*),iii)lowpopulationwithmoderate DI (*A. pauciflorum*), and iv) low populationandlow DI(*P. speciosa*and*A. auriculiformis*).~~

In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) wassignificantlyhigherthanotherplantsforeachdiseasescore.Thispatternwascommonwherehighdiseasescoreswerealso foundinthehighpopulation. ….. reportedthatsusceptiblelettucecultivarsshowedhigh*Fusarium*populationlevelsand ~~the~~vulnerableblackbeangenotypeshowedapopulationlevelof15.4×10^5CFU^{g-1}offreshweight(.Inthesecondpattern,thepopulationwasmoderateinamoderatesusceptiblehost(*L.leucocephala*).Thesimilarresultwasalso~~Thisalso~~ occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a~~fairly~~ significantlyhighernumber of pathogens on the roots…. .

A special pattern occurred on *A. pauciflorum*that could have been caused by a moderate pathogen infection, but the pathogenpopulationwaslow.Thismightbedueotheplant'sdefencemechanism.~~Severalstudiesonresistantcultivars havecorroboratedthis;,forexample,~~ …… reportedthatresistantpepperplantsalsosupport~~the~~pathogen's development inroots,evenwithoutexternalsymptoms.Similarphenomena~~were~~was reportedby ……. whonotedthatsometomato plants~~couldbe~~areinfectedmoderately(score1-2)by *F.oxysporum*f.sp. *lycopersici*,butthepopulationwaslowerthan~~the~~other plants in ~~the~~same score.

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The~~pathogen~~infectionandtotalpopulationon*Parkiaspeciosa*and*A.auriculiformis*waslowerandthetotal ~~populationwasalsolow~~.Thisindicatedtha~~t~~thepla~~nt~~s~~b~~elongedtotheresistantplantgroup. ……. reportedthatwhen resistantstrawberryplantswereinoculatedwith*F.oxysporum*f.sp.*fragariae*,thecultivarformedabarrierwith accumulatedphenoliccellsinthehypodermalla yerthateffectivelylimitsthepathogens'colon~~i~~sationandprevents~~t~~heepenetrati invasionofrootvascularlissue.If~~Ther~~thetissu nbyhyphaewaslimitedtotheepidermisthenthe,andth pathogens~~did~~donotreachthevascularlissue. Also,theresultsofastudyconducted by… …reported~~on~~thatbanana clonestolerantto*F.oxysporum*f.sp.*cubense*correspondwiththis,withasignificantincreaseintheinductionofcell wall-associatedphenoliccompounds.~~Moreover,~~…….alsoreportedthat*Fusariumoxysporum*f.sp.*ciceris*race0 remainedintheintercellularspaceofthe~~root~~cortexandfailedtoreachthexylemin~~res~~istantchickpeacultivars.

Inthisstudy,*A.crassicarpa*and*F.moluccana*wereproventobeanalternativehostof*F.oxysporum*. ~~Meanwhile~~,Whereas*L.leucocephala*,*A.pauciflorum*,*P.speciosa*,and*A.auriculiformis*hadpotentialasalternativehosts. Manyplantsofthe~~Fabaceae~~fabaceaefamily~~were~~wasattackedbyformaespecialis*F.oxysporum*,suchas,*Vigna angularis*(*F.oxysporum*f.sp.*adzukicola*),*Cicerarietinum*,*Cicer*spp.(*F.oxysporum*f.sp.*ciceris*),*Acacia*spp.(*F. oxysporum*f.sp.*koae*),*Lensculinaris*,*L.esculenta*(*F.oxysporum*f.sp.*lentis*),*Medicagosativa*(*F.oxysporum*f.sp. *medicaginis*),*Phaseolusvulgaris*,*P.coccineus*(*F.oxysporum*f.sp.*phaseoli*),*Pisumsativum*,*Cicerarietinum*(*F. oxysporum*f.sp.*pisi*),and~~others~~ …However,inthisstudy,*F.oxysporum*isolatedfrom*A.mangium*hasawidehost rangefrom~~the~~Fabaceae~~fabaceae~~family;therefore,itisnotclassifiedasformaespecialis.

Inconclusion,*F.oxysporum*isolatedfrom*A.mangium*causes~~disease~~infectioninseveraltypesofforestandindustrial plants.~~Therefore,since~~Sinceithasawidehostrange,itisnotclassifiedaspartoftheformaespecialisgroup.

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Answer Review1

Hostrangestudiesof*Fusariumoxysporum*, causalagent of seedlingwilt diseaseof*Acaciamangium*

Abstract.*Fusariumoxysporum*isaseriouspathogenthatcausesseverewiltdiseaseincommercialnurseriesof*Acaciamangium*in SouthSumatra.Thisstudyaimedtoinvestigatethehostrangeof*F.oxysporum*asanurserywiltpathogenin*A.mangium*andseveral forestandindustrialplants.Threeisolatesof*F.oxysporum*withdifferenttranslationelongationfactor(*tef*1-α)sequencesweretested forpathogenicityondifferentfabaceaefamilyplantsandthegrowthofpopulationwasalsoobserved.Theresultsshowedthatallthe threeisolateswereabletoinfectallthetestedplantswithdifferentreactionsofwiltdisease.The*Acaciacrassicarpa*and*Falcataria moluccana*werehighlysusceptible;*Archidendronpauciflorum*,*Leucaenaleucocephala*,and*Parkiaspeciosa*weremoderately vulnerableand*Acaciaauriculiformis*wasmoderatelyresistant.Thepathogenpopulationin*A.crassicarpa*and*F.moluccana*grew rapidlyalongwiththeincreaseindiseasescores,whilein*L.leucocephala*itwasmoderate,andslowin*A.pauciflorum*,*P.speciosa*and *A. auriculiformis*plants.Inconclusion,*F. oxysporum*pathogen,whichwas isolatedfrom*A.mangium*,has awiderange of hostsinthe fabaceaefamily.

Keyword:*Acaciamangium*,fabaceae,*Fusariumoxysporum*,hostrange,seedling wilt

INTRODUCTION

Acaciamangium(Willd.....)isaspeciesofplantthatoriginatedinseveralregionsofIndonesia,PapuaNewGuinea, andAustralia,andwhich,hasalsobeenfoundforafewdecadesinthehumidtropicallowlandsofAsia,SouthAmerica, andAfrica(KoutikaandRichardson2019(....)).Itisplantedonalargescaleforindustrialpurposesandforestrestoration inthetropics (MatsumuraandNaoto2011). Sincethisplantspeciesisknownforitsfastgrowthandhighadaptabilityto variousenvironmentalconditions (Asifetal.2017), itiswidelyusedforagroforestry,forestry,andrestorationofdegraded land(KoutikaandRichardson2019).

*Fusariumoxysporum*isanimportantpathogenicfungusthatcauseswiltdiseaseindifferentplantsallovertheworld. reportedthatitwasidentifiedasthecausativeagentofvascularwiltinseveralcommercial nurseriesof*A.mangium*inSouthSumatra.Themainsourceoftransmissionisthroughinfectedseedlingsandsoil,which isrelativelydifficulttotreataftercontamination.Thefungussurvivesbyformingchlamydosporesthatallowittolivefora longtime,evenwithoutahostplant (Ignjatovetal.2012;Koyyappurathetal.2016;Ranaetal.2017). Furthermore,it attacksalmosteverytypeofplant,fromcultivatedtoforestandwild(e.g.weeds)(Joshi2018).Thisfungusisalsoableto attackvariousplanthabitssuchastrees(Zhangetal.2013),herbaceousplants(JacobsandHeerden2012),andvines (Rooney-LathamandBlomquist2011).Severaltypesofforestplantsthathavereportedlybeenattackedby*F.oxysporum* are*Pinusmassoniana* (LuoandYu2020), *Tectonagrandis* (Borgesetal.2018) *Pseudotsugamenziesii* (Stewartetal. 2011),*Acaciamangium*(Widyastutietal.2013)andothers.(.....).

Since*F.oxysporum*hasahighlevelofhostspecificity,itisclassifiedasaformaespecies (Burkhardtetal.2019; Tayloretal.2019) ..... According to LeslieandSummerell(2006)....., morethan100formaespeciesandraceshavebeen identifiedandarewidespreadintheworld.

Besides*A.mangium*,whichisthemainplantofindustrialforestryinIndonesia,otherplants,suchas*Acacia crassicarpa*,*Acaciaauriculiformis*,*Parkiaspeciosa*,*Archidendronpauciflorum*,*Falcatariamoluccana*,and*Leucaena leucocephala*arealsoimportantandhavehigheconomicvalue.Consideringthattheybelongtothesamefamily (Fabaceae),theycanbecomethemainoralternativehostsfor*F.oxysporum*,causativeagentofwiltdisease.Thisstudy aimedtoinvestigatethehostrangeof*F.oxysporum*asanurserywiltpathogenin*A.mangium*andseveralindustrialand localforestplantsinIndonesia.

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MATERIALSANDMETHOD

Fungalisolates

Threepathogenicisolatesof*F.oxysporum*(AF01,BF05,andDF11),wereselected,whichweredifferentiated accordingtotheir*tef*1-αsequence(,wereutilised;theywerechosenbecauseapreviousstudy(haddescribedthemasthe mostpathogenictoA.*mangium*(Figure1).IsolateswereculturedonPDBliquidmedium(potatodextrosebroth)and incubatedat26-28°Conashaker(150rpm)foraboutfivedays.Thenthemyceliasuspensionwasfilteredusingtwo layersofsterilegauzetoseparatetheconidiaandhyphae.Theconidialconcentrationwasdeterminedusinga hemocytometer and then adjusted to a concentration of 10^6 ml^{-1} for pathogenicity test.

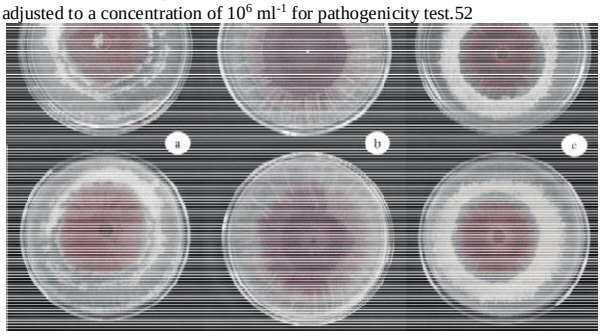

Figure1.*F.oxysporum*isolatesonPDAmedium.(a)AF01,(b)BF05,and(c)DF11.Firstline:Frontview;secondline:reverseview.

Plantmaterial

Theplantsusedweremembersofthefabaceaefamily,namely*A.crassicarpa*,*A.auriculiformis*,*F.moluccana*,*A. pauciflorum*,*P.speciosa*,and*L.leucocephala*,whichwereonemonthold.TheseedlingswereobtainedfromtheForest CropsResearchInstitute,SouthSumatra.Seedlingsweretransferredinamixedmediumwithcocopeat(1:1)usinga plasticpotof10cmdiameterand10cmheight,andthenplacedinashadehouse.

Pathogenicitytest

Apathogenicitytestwascarriedoutusingrootdipmethod,inwhichtherootswerewashedunderrunningwaterand thenimmersedin250mlofconidiasuspension(10^6conidiaml^{-1})for15minutes.Thecontrolplantswereimmersedin steriledistilledwater,andtheseedlingsweretransplantedintoplasticpotsandplacedunderahouseshade.Eachisolate wasinoculatedon25plantswithfivereplicates(fiveplantsper-replicate).Then,diseaseseveritywascalculatedusingthe methoddesignedby Muslimetal.(2003a) andmodifiedusingadiseaseindex(DI)0-4,where0=nodisease/healthy seedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severewilt,and4=deadseedling.Furthermore, plantresponseswereregroupedas,R=resistant(DI=0),MR=moderatelyresistant/tolerance(DI=<1),MS=moderately susceptible(DI=1.0-2.0),S=susceptible(DI=2.1-3.0)andHS=highlysusceptible(DI=3.1-4.0).Thedevelopmentof diseasewasobserved1–21daysafterinoculation.

***Fusariumoxysporum*population**

ThepopulationofF.*oxysporum*intherootswascalculatedattheendoftheexperimentusingthemethodof(Muslim etal.2003b;Lietal.2008;Horinouchietal.2011)withmodificationstothesurfacesterilizationofsamples.Thenthe plantsweregroupedaccordingtoseverity(diseasescore)andwashedseparatelyunderrunningwatertoremovesoil residues.Afterthat,allplantsineachscoreweresurfacesterilisedusing1%sodiumhypochloritefor15minutes,then rinsedthreetimeswithdistilledwater.Thesamplesandwater(1:100w/v)werehomogenisedusingblenderat8000rpm for10minutes.Thentheywerefilteredusingtwolayersofsterilegauzeanddiluted10to1000times.Thesuspensionwas spreadonPeptonePCNBagarmMedia(PPA/NashSnyderMedium)(LeslieandSummerrel,2006)intriplicate(fivePetri dishesperreplication)andincubatedindarkforsevendaysatroomtemperature.Thenumberofcolony-formingunits (CFU)of*F.oxysporum*wascalculatedonthebasisoffreshweightpergramofsampleandgroupedaccordingtothe diseasesseveritylevel.recordedbasedonthediseaseseverity.

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RESULTSANDDISCUSSIONN

Pathogenicitytest

Theresultsshowedthatallthesixforestplantstestedhadsimilarreaction~~t~~othepathogen.Sevendaysafter inoculation,allthe plantsshowedtypicalsymptomsof*F.oxysporum*infection,i.e.yellowingofoldestleavesclosesttothe stembase,whichgraduallyprogresstoyoungershoots,severewilting,drying,fallingofleaves,andeventuallyplantdie. Anothersymptomthatappearedwassuddenwiltinganddeathofplantwithoutchangingtheleafcolour,whilecontrol plantsdidnotshowanysymptoms(Figure2,3,4,5,6&7).

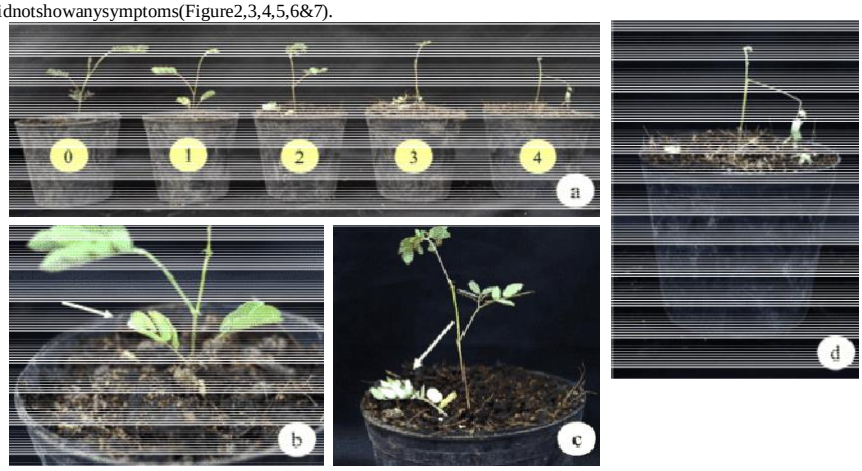

Figure2. DiseaseindexofA*cacia crassicarpa*(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initialsymptoms: yellowingfromoldestleaves;(c)advancedsymptoms:fallingleaves;(d)deadplant.

Figure3. Diseaseindex on*Falcatariamoluccana*(a)from left: healthy plantto 100%wiltedleaves(score0−4); (b) initialsymptoms: yellowingfromoldestleaves;(c)advancedsymptoms:curved,dry,andfallingleaves;(d)deadplant.

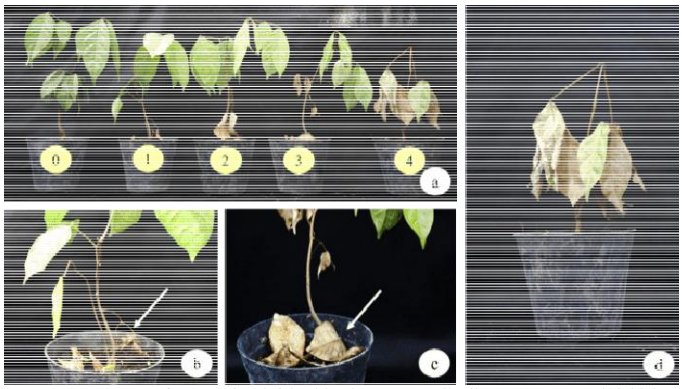

Figure4. Disease index on *Archidendron pauciflorum* (a) from left: healthy plant to 100% wilted leaves (score 0−4), (b) initial symptoms: yellowing and dry from oldest leaves, (c) advanced symptoms: falling leaves, (d) dead plant.

Figure5. Disease index on *Leucaena leucocephala* (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward.

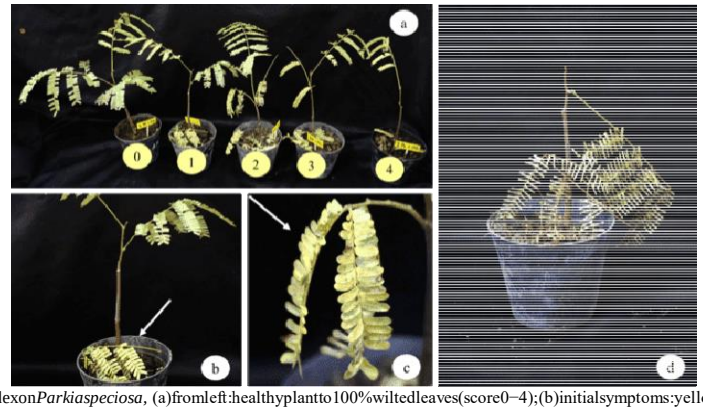

Figure6. Disease index on *Parkia speciosa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant.

Figure7. Diseaseindexon*Acaciaauriculiformis*,~~(a)~~fromleft:healthyplantto~~100%~~wilted~~leaves~~anddeadplant(score0and ~~–~~4));(band c) advanced symptoms: ……..

Diseaseseveritywassignificantlyhigherthancontrols. *A.crassicarpa*and *F.moluccana*weremostseverelyaffectedwithanaveragescoreof4.00and3.44,respectively~~andtheincidenceofwiltingwas100%~~.Onth eotherhand, *A.pauciflorum*, *L.leucocephala*,and *P.speciosa*wereshowedmoderatediseaseseverityi.e.1.96,1.68,and1.80,respectively,whereas *A.auriculiformis*hadthelowest(0.36)diseaseseverity(Table1)and(16%)incidence)(Table1).Basedonthediseasescore,hostplantswer eclassifiedintothreegroups:i)highlysusceptible(*A.crassicarpa*and *F.moluccana*),ii)moderatelysusceptible(*A.pauciflorum* *P.speciosa*,and *L.leucocephala*),andiii)moderateresistance/tolerance (*A. auriculiformis*) (Table 1).

Resultexhibitedthattherewasnosignificantdifferencebetweenthediseaseseverityinthesamehostthathadbeeninoculated with different isolates.

Table1.~~Pathogenicityand~~diseaseseverityandhostresponsestoo~~f~~*Fusariumoxysporum*isolatedfrom*Acaciamangium*

Plantspecies	Isolates[a)]					
	AF01[b)]Response		[c)]BF05Response		DF11Response	
Acaciacrassicarpa	4.00a	HS[c)]	3.48 a	HS	3.96a	HS
Falcatariamoluccana	3.44ab	HS	3.04 a	HS	2.80ab	S
Archidendronpauciflorum	1.96bc	MS	1.88 b	MS	1.40cd	MS
Leucaenaleucocephala	1.52c	MS	1.56 b	MS	1.68bc	MS
Parkiaspeciosa	1.80c	MS	1.04 bc	MS	2.16bc	S
Acaciaauriculiformis	0.36d	MR	0.40 c	MR	0.60d	MR

Valuesfollowedbythesameletterineachrowarenotsignificant.

[a] DI0-4,where0=nodisease/healthyseedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severewilt, and4=deadseedling.

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[b] *F.oxysporum* isolates.

[c] Host response were grouped as: R= resistant (DI=0); MR=moderately resistant/tolerance (DI=<1); MS=moderately susceptible (DI=1.0-2.0); S=susceptible (DI=2.1-3.0); HS=highly susceptible (DI=3.1-4.0) (Bertetti et al. 2018)

***Fusarium oxysporum* population**

The total population of *F.oxysporum* on the roots was determined by calculating the CFU for each category of damage. The results showed that at a score of 4, *A. crassicarpa* and *F. moluccana* showed a significantly higher population (82.00–105.10 × 10^4 CFU g^{-1} fresh weight) than other plants. The lowest population was recorded in *P. speciosa* and *A.pauciflorum* (3.57–12.27 × 10^4 CFU g^{-1} fresh weight). This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A.Auriculiformis* for DI 2 and 3. In DI 1, the highest population was recorded in *F.moluccana* and *L. leucocephala*, while *A.crassicarpa* and *A.auriculiformis* had no sample for DI 1. In ~~control plants (~~DI=0), the population was significantly higher in *L.leucocephala* and *A.auriculiformis* and no sample was noted in *A.crassicarpa* and *F.moluccana* for DI 0 (Table 2 & Table 3).

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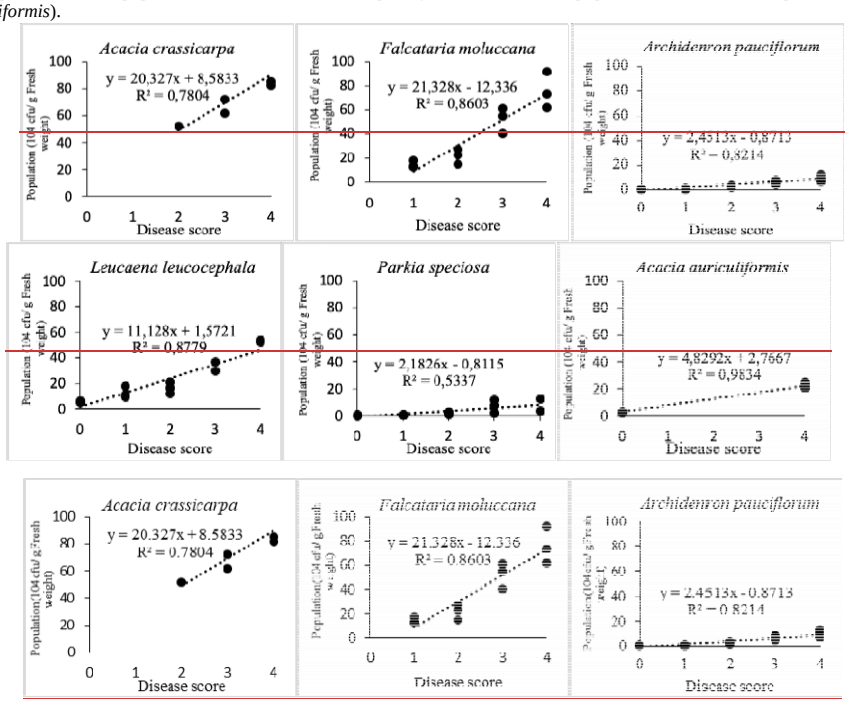
The regression analysis results showed that all plants except *P.speciosa* had a linear relationship pattern between the increase in disease score and population. The pathogenic population on *A.crassicarpa* and *F.moluccana* grew rapidly along with the increase in disease scores, as indicated by the magnitude of regression gradient coefficient (m=20.3–21.3). However, moderate increase was observed in *L.leucocephala* (m=11.2) (m=11.2) and very slow in *A.pauciflorum*, *P.speciose*, and *A. auriculiformis* (m=2.2–4.8) (Figure 8).

Table 3 showed that isolates were different in *tef1-α*, but the population and DI patterns were similar for each test plant. The correlation between the population of pathogen (g^{-1} fresh weight) and the level of DI were described as follows: i) high pathogen populations with high DI (*A.crassicarpa* and *F.moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population with moderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).



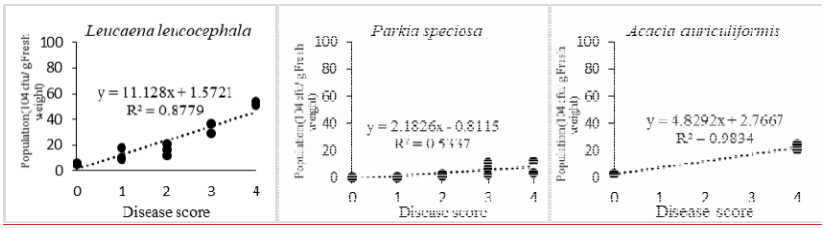


Figure8. Regressionanalysisofdiseasescorerateand*F.oxysporum*population

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Table 2. *Fusarium oxysporum* population onrootineachdiseaseindex

Plantspecies	Populationof*Fusariumoxysporum*(×10^4CFU/gfreshweight)[a]					Average[c]
	0[b]	1	2	3	4	
AF01[d]						
Acaciacrassicarpa	n.s	n.s	n.s	n.s	85.13a[e]	85.13
Falcatariamoluccana	n.s	17.,77a	22.77a	60.98a	91.87a	76.50
Archidendronpauciflorum	0.45b	1.10b	3.22b	8.15b	12.53cd	5.06
Leucaenaleucocephala	6.17a	18.10a	20.93a	n.s	51.67b	22.13
Parkia speciosa	0.32b	0.45b	2.58b	7.27b	3.57d	2.16
Acaciaauriculiformis	2.92a	n.s	n.s	n.s	24.53c	4.65
BF05						
Acaciacrassicarpa	n.s	n.s	51.80a	72.08a	105.10a	92.61
Falcatariamoluccana	n.s	13.22a	15.32b	40.33b	61.67b	43.85
Archidendronpauciflorum	0.47c	0.63b	1.73c	6.88c	9.90d	3.60
Leucaenaleucocephala	4.67a	9.02a	12.32b	29.32b	n.s	11.16
Parkia speciosa	0.48c	0.57b	1.27c	2.33d	n.s	0.87
Acaciaauriculiformis	2.55b	n.s	n.s	n.s	20.43c	3.98
DF11						
Acaciacrassicarpa	n.s	n.s	n.s	61.92a	82.00a	81.20
Falcatariamoluccana	n.s	12.50a	27.47a	54.93a	73.00a	47.93
Archidendronpauciflorum	0.35c	0.35b	3.37c	4.42c	6.92e	2.19
Leucaenaleucocephala	5.58a	11.17a	16.53b	36.63b	54.27b	19.69
Parkia speciosa	0.25c	0.48b	1.58c	11.97d	12.27d	5.79
Acaciaauriculiformis	2.83b	n.s	n.s	n.s	21.28c	5.05

n.s:No sample,cfu:colonyformingunit

[a] *F. oxysporum* populationscalculatedattheend oftheexperiment(21 daysafterinoculation).

[b] DI0-4;0=nodisease/healthyseedling;1=yellowleaves;2=yellowleavesandslightlywilted;3=severewilt;and4=dead seedling.

[c] Averageof*F.oxysporum*population(cfu/gfreshweight)=($P_0A+P_1B+P_2C+P_3D+P_4E$)/N;wherеP0,P1,P2,P3,andP4=populationofpathogeninscore0,1,2,3,and4:A=numberofplantsonscore 0;B=numberofplants onscore1; C=numberofplantsonscore2;D=number ofplantsonscore3;E=numberof plants on score4; N= totalnumberofplants.

[d] *F.oxysporum*isolates

[e] Valuesfollowed bythesameletterineachrowarenot significant.

Table3. *Fusarium oxysporum* population average and disease index of plant

Plant species	Population average ($\times 10^4$ CFU/g fresh weight)[a]			Disease index[b]		
	AF01[c]	BF05	DF11	AF01	BF05	DF11
Acacia crassicarpa	85.13	92.61	81.20	4.00	3.48	3.96
Falcataria moluccana	76.50	43.85	47.93	3.44	3.04	2.80
Archidendron pauciflorum	5.06	3.60	2.19	1.96	1.88	1.40
Leucaena leucocephala	22.13	11.16	19.69	1.52	1.56	1.68
Parkia speciosa	2.16	0.87	5.79	1.80	1.04	2.16
Acacia auriculiformis	4.65	3.98	5.05	0.36	0.40	0.60

[a] Average of *F. oxysporum* population (cfu/g fresh weight) = $(P_0A+P_1B+P_2C+P_3D+P_4E)/N$; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.

[b] DI 0-4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] *F. oxysporum* isolates.

Discussion

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mMangium* in South Sumatra (Solehaet al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information about plant species that have the potential to become alternative host or main hosts for the pathogen (Sampaio et al. 2021).

The results indicated that *F. oxysporum*, which causes vascular wilt in *A. mangium* nursery, can also infect fabaceae plants with various host responses. *A. crassicarpa* and *F. moluccana* were highly susceptible, while *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen caused wilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in *A. auriculiformis*, but it had the potential to damage plants. *F. oxysporum* able to infect plant seven with a low DI, causing the death of cultivars. Moreover, when a plant is grown in contaminated soil, there is a high risk of damage to crops. A similar incident was reported by Pastrana et al. (2017) ……, in which *F. oxysporum* from black berry also caused sudden death in strawberries.; therefore, it becomes a threat when planted on adjacent land. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot also causes disease so in Euphorbia (Bertetti et al. 2017). when cultivated on the same land…….

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative host and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrence of new outbreaks because the pathogenic strains adapted to the soil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the pathogens proliferation and adaptation to other plants.

The pathogen population in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing disease scores, while in *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* grew slowly. In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern was common where the population of the pathogen was also high disease scores Scott et al. (2014) This pattern was common where high disease scores were also found in the high population reported that susceptible lettuce cultivars showed high *Fusarium* population level and vulnerable black bean genotypes showed a population level of 15.4×10^5 CFU $^{g-1}$ (de Borba et al. 2017). In The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate within a moderate a moderate disease score. susceptible host (*L. leucocephala*). The similar result was also occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a significantly moderate higher number of pathogens on roots (Molinero-Ruiz et al. 2011)…… .

A special pattern occurred on *A. pauciflorum* that could have been that *F. oxysporum* caused by a moderate pathogen infection, but the pathogen population was low. This might be due to the plant defence mechanism. Scott et al. (2014)…… …

…. reported that resistant pepper plants also support pathogen development in roots, even without external symptoms. Similar phenomena was reported by Muslim et al. (2003a) ……. who noted that some tomato plants are infected moderately (score 1-2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower than other plants in same score.

The infection and total population on *Parkia speciosa* and *A. auriculiformis* was lower. This indicated that plant belonged to the resistant plant group. Fang et al. (2012) ……. reported that when resistant strawberry plants were inoculated with *F. oxysporum* f. sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the

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hypodermallayerthateffectivelylimits thepathogencolonisationandpreventtheinvasionof root vasculartissue. If thetissuepenetrationbyhyphaewaslimitedtothe~~epidermis~~epidermis,thenthepathogensdonotreachthevasculartissue.VanDenBergetal.(2007)~~.....~~reportedthatbananaclonestolerantto *F.oxysporum*f.sp. *cubense*correspondwiththis,withasignificantincreaseintheinductionofcellwall-associatedphenoliccompounds.Jiménez-Fernándezetal.(2013) ~~.....~~.alsoreportedthat *Fusariumoxysporum*f.sp. *ciceris*race0remainedintheintercellularspaceofrootcortexandfailed to reach xylem in resistant chickpea cultivars.

Inthisstudy, *A.crassicarpa*and *F.moluccana* wereproventobeanalternativehostof *F. oxysporum*.Whereas *L.leucocephala*, *A.pauciflorum*, *P.speciosa*,and *A.auriculiformis*hadpotentialasalternativehosts.Manyplantsoffabaceae family was attacked byformae specialis *F. oxysporum*, suchas *Vigna angularis* (*F. oxysporum* f.sp. *adzukicola*),*Cicerarietinum*,*Cicer*spp.(*F.oxysporum* f.sp. *ciceris*), *Acacia*spp.(*F.oxysporum* f.sp. *koae*), *Lensculinaris*,*L.esculenta*(*F.oxysporum* f.sp. *lentis*), *Medicagosativa*(*F.oxysporum* f.sp. *medicaginis*), *Phaseolusvulgaris*, *P.coccineus*(*F.oxysporum* f.sp. *phaseoli*), *Pisumsativum*,*Cicerarietinum*(*F.oxysporum* f.sp. *pisi*)(Edel-HermannandLecomte2019)~~....~~.However,inthisstudy, *F.oxysporum*isolatedfrom*A.mangium*hasawidehostrangefromfabaceaefamily; therefore, it is not classified as formae specialis.

Inconclusion,*F.oxysporum*isolatedfrom*A.mangium*causesinfectioninseveraltypesofforestandindustrialplants. Sinceithasawidehostrange,itisnotclassifiedaspartoftheformaespecialisgroup.

ACKNOWLEDGEMENT

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Round2Status

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[biodiv]EditorDecision	2021-11-17 06:12AM
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[biodiv]EditorDecision	2021-12-04 05:51AM
[biodiv]EditorDecision	2021-12-16 03:36AM
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Reviewer'sAttachments

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Name	From	LastReply	Replies	Closed
Manuscript Revision 9450-52530-1-5-20211117	amuslim 2021-11-26 01:25 AM	-	0	
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3. Bukti konfirmasi review kedua dari reviewer

a.muslimunsri<a_muslim@unsri.ac.id>

[biodiv]EditorDecision

2messages

Smujo Editors <smujo.id@gmail.com> Mon, Nov 29, 2021 at 6:25 AM
To: Soleha Soleha <soleha057@gmail.com>, Ahmad Muslim <a_muslim@unsri.ac.id>, Suwandi Suwandi <Suwandi@fp.unsri.ac.id>,SabaruddinKadir<sabar@pps.unsri.ac.id>,RahmatPratama <rahmatpratama@pps.unsri.ac.id>

SolehaSoleha,AhmadMuslim,SuwandiSuwandi,SabaruddinKadir,RahmatPratama:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host Range Studies of Fusarium oxysporum, the Causal Agent of Seedling Wilt Disease of Acacia mangium Willd.".

Ourdecisionis:RevisionsRequired

ReviewerA:

Comments:

1. Therearestillsomecorrectionsinpaper,pleasemakecorrectionaspersugesstion.

2. Pleaseclearthemeaningofnosamples.

3. readpaperverycarefully.

Recommendation:SeeComments

BiodiversitasJournalofBiologicalDiversity

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4. Bukti konfirmasi revision of manuscript kedua, respon kepada reviewer dan perbaikan revisi artikel yang di resubmit

a. muslim unsri <a_muslim@unsri.ac.id> Thu, Dec 2, 2021 at 10:05 AM
To: Smujo Editors <smujo.id@gmail.com>

DearEditorinChief
BiodiversitasJournalofBiologicalDiversity

Thank you very much for your email regarding reviewer's recommendation, suggestion, and revision of our manuscript. We would like to thank and appreciate for all reviewers'suggestions and corrections.

We have made corrections and some modification according to Reviewer's revisions. Here, we enclose ourrevisedmanuscriptwithtrackedchangesofthemanuscriptentitled"HostRangeStudiesof *Fusarium*

oxysporum,CausalagentofSeedlingWiltDiseaseof*Acaciamangium*"bySolehaSoleha,Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama.

In this opportunity, we would like to re-submit our revised manuscript for publication inBiodiversitasJournal of Biological Diversity.

Belowisasummaryofourchangesmadeinresponsetothereviewer'scomments.

1. ***Reviewer'scomment:***Therearestillsomecorrectionsinpaper,pleasemakecorrectionaspersugesstion.
Our response: We have changed the word and the sentences, that some minor corrections arechanged directly in the manuscript and some corrections are as follows.

Reviewer'scomment:Ifyouhavefollowedanyreferencefordiseaseindexpleasementiontheauthor

Ourresponse:wehaveinsertreference(Bertettietal.2018)fordiseaseindexinthis line.

Reviewer'scomment:Sstandsforsusceptiblebutinthetextyouhaveclassified*Falcatariamoluccana* highlysusceptiblehost.Pleasecheckitagain.
Ourresponse: Weclassified*Falcatariamoluccana*as highly susceptible host because this plantshowed highly susceptible (HS) responses against two isolates (AF01 and BF05) and only one isolate (DF11) show susceptible response (S).

Reviewer'scomment:Sstandsforsusceptiblebutinthetextyouhaveclassified*Parkiaspeciosa* moderatesusceptiblehost.Pleasecheckitagain.
Our response: We classified*Parkia speciosa* as moderate susceptible host because this plantshowed moderate susceptible (MS) responses against two isolates (AF01 and BF05) and only one isolate (DF11) shows susceptible response (S).

Reviewer'scomment:Removetheoldfigures
Ourresponse: Wehaveremovedtheoldfigures

2. ***Reviewer'scomment:***Pleaseclearthemeaningofnosamples.
Ourresponse:
Reviewer'scomment:Ihaveaskedyouearlierthatwhatdoyoumeanbynosamples.Doesitmeanthat *Fusaium*wasnotfoundin*A.auriculiformis*.Pleaseclearit.
Our response: No sample means that there was not found on inoculated*Acacia auriculiformis*that showed disease index 2 and 3. All inoculated plants showed only disease index 0 and disease index 4. In thisexperimentweobservedpopulationof*Fusariumoxysporum*basedondiseaseindexofthe inoculated plant.

Reviewer's comment: Again the same question that I have asked you earlier that what do you meanby no samples. Does it mean that*Fusaium*was not found in*A. Auriculiformis*and*A. crassicarpa*.Please clear it.
Ourresponse: Nosamplemeansthattherewasnotfoundoninoculated *A.auriculiformis*and *A. crassicarpa* that showed disease index 1. All inoculated*A. auriculiformis* showed only diseases index0 and 4. While*A.crassicarpa* showed only disease index 4 (AF01); disease index 2, 3, and 4 (BF05);and disease index 3 and 4 (DF11). In this experiment we observed population of*Fusarium oxysporum*based on disease index of the inoculated plant.

Reviewer'scomment:Confusing line "In control plants (DI=0), the population was significantly higher in *L. leucocephala* and*A. auriculiformis*and no sample was noted in*A. crassicarpa* and*F.moluccana* (Table 2 & Table 3)."
Ourresponse:*Fusariumoxysporum*wasnotfoundoncontroluninoculatedplants. DI0meansthatdiseaseindexforinoculated*L.leucocephala,Leucaenaleucocephala,Parkiaspeciosa* and*A.auriculiformis.*While,*A.crassicarpa*and*F.moluccana*didnotproduceDI0(Itismeanthatthere

wasnosampleforA.*crassicarpa*and*F.moluccana*), because all plants showed symptoms (Table 2). W have change "control plants" to be "inoculated plants"

3. ***Reviewer'scomment:***readpaperverycarefully.
Our response: We have read our paper carefully and we changed all the words/sentences as reviewer' comment, suggestion and revision.

-
We feel that these changes have adequately addressed the comments and suggestions of the reviewers,and we look forward to publication in the Biodiversitas Journal of Biological Diversity.

Pleasefeelfreetocontactmeifyouneedanyadditionalinformationorclarification. Thank you very much for your excellent cooperation

Sincerely,
AhmadMuslim
Senior lecturer
FacultyofAgriculture,SriwijayaUniversity
Jl.Palembang-PrabumulihKm.32,Indralaya,Palembang,Indonesia E-mail: a_muslim@unsri.ac.id

[Quotedtexthidden]

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Review2

Hostrangestudiesof*Fusariumoxysporum*, causalagent of seedlingwilt diseaseof*Acaciamangium*

Abstract.*Fusariumoxysporum*isaseriouspathogenthatcausessevere wiltdiseaseincommercialnurseriesof*Acaciamangium*in SouthSumatra.Thisstudyaimedtoinvestigatethehostrangeof*F.oxysporum*asanurserywiltpathogenin*A.mangium*andseveral forestandindustrialplants.Threeisolatesof*F.oxysporum*withdifferenttranslationelongationfactor(*tef*1-α)sequencesweretested forpathogenicityondifferentfabaceaefamilyplantsandthegrowthofpopulationwasalsoobserved.Theresultsshowedthatallthe threeisolateswereabletoinfectallthetestedplantswithdifferentreactionsofwiltdisease.The*Acaciacrassicarpa*and*Falcataria moluccana*werehighlysusceptible;*Archidendronpauciflorum*,*Leucaenaleucocephala*,and*Parkiaspeciosa*weremoderately vulnerableand*Acaciauriculiformis*wasmoderatelyresistant.Thepathogenpopulationin*A.crassicarpa*and*F.moluccana*grew rapidlyalongwiththeincreaseindiseasescores,whilein*L.leucocephala*itwasmoderate,andslowin*A.pauciflorum*,*P.speciosa*and *A. auriculiformis*plants.Inconclusion,*F. oxysporum*pathogen,whichwas isolatedfrom*A.mangium*,has awiderange of hostsinthe fabaceaefamily.

Keyword:*Acaciamangium*,fabaceae,*Fusariumoxysporum*,hostrange,seedling wilt

INTRODUCTION

Acaciamangium(Willd.,...)isaspeciesofplantthatoriginatedinseveralregionsofIndonesia,PapuaNewGuinea, andAustralia,andwhich,hasalsobeenfoundforafewdecadesinthehumidtropicallowlandsofAsia,SouthAmerica, andAfrica(KoutikaandRichardson2019(....)).Itisplantedonalargescaleforindustrialpurposesandforestrestoration inthetropics (MatsumuraandNaoto2011). Sincethisplantspeciesisknownforitsfastgrowthandhighadaptabilityto variousenvironmentalconditions (Asifetal.2017), itiswidelyusedforagroforestry,forestry,andrestorationofdegraded land(KoutikaandRichardson2019).

*Fusariumoxysporum*isanimportantpathogenicfungusthatcauseswiltdiseaseindifferentplantsallovertheworld. Sridhar(2016)reportedthatitwasidentifiedasthecausativeagentofvascularwiltinseveralcommercial nurseriesof*A.mangium*inSouthSumatra.Themainsourceoftransmissionisthroughinfectedseedlingsandsoil,which isrelativelydifficulttotreataftercontamination.Thefungussurvivesbyformingchlamydosporesthatallowittolivefora longtime,evenwithoutahostplant (Ignjatovetal.2012;Koyyappurathetal.2016;Ranaetal.2017). Furthermore,it attacksalmosteverytypeofplant,fromcultivatedtoforestandwild(e.g.weeds)(Joshi2018).Thisfungusisalsoableto attackvariousplanthabitssuchastrees(Zhangetal.2013),herbaceousplants(JacobsandHeerden2012),andvines (Rooney-LathamandBlomquist2011).Severaltypesofforestplantsthathavereportedlybeenattackedby*F.oxysporum* are*Pinusmassoniana* (LuoandYu2020), *Tectonagrandis* (Borgesetal.2018) *Pseudotsugamenziesii* (Stewartetal. 2011),*Acaciamangium*(Widyastutietal.2013)andothers.(.....).

Since*F.oxysporum*hasahighlevelofhostspecificity,itisclassifiedasaformaespecies (Burkhardtetal.2019; Tayloretal.2019) ..... According to LeslieandSummerell(2006)....., morethan100formaespeciesandraceshavebeen identifiedandarewidespreadintheworld.

Besides*A.mangium*,whichisthemainplantofindustrialforestryinIndonesia,otherplants,suchas*Acacia crassicarpa*,*Acaciauriculiformis*,*Parkiaspeciosa*,*Archidendronpauciflorum*,*Falcatariamoluccana*,and*Leucaena leucocephala*arealsoimportantandhavehigheconomicvalue.Consideringthattheybelongtothesamefamily (Fabaceae),theycanbecomethemainoralternativehostsfor*F.oxysporum*,causativeagentofwiltdisease.Thisstudy aimedtoinvestigatethehostrangeof*F.oxysporum*asanurserywiltpathogenin*A.mangium*andseveralindustrialand localforestplantsinIndonesia.

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MATERIALSANDMETHOD

Fungalisolates

Threepathogenicisolatesof*F.oxysporum*(AF01,BF05,andDF11),wereselected,whichweredifferentiated accordingtotheir*tef*1-αsequence(,wereutilised;theywerechosenbecauseapreviousstudy(haddescribedthemasthe mostpathogenictoA.*mangium*(Figure1).IsolatesweretulturedonPDBliquidmedium(potatodextrosebroth)and incubatedat26-28°Conashaker(150rpm)foraboutfivedays.Thenthemyceliasuspensionwasfilteredusingtwo layersofsterilegauzetoseparatetheconidiaandhyphae.Theconidialconcentrationwasdeterminedusinga hemocytometer and then adjusted to a concentration of 10^6 ml^{-1} for pathogenicity test.

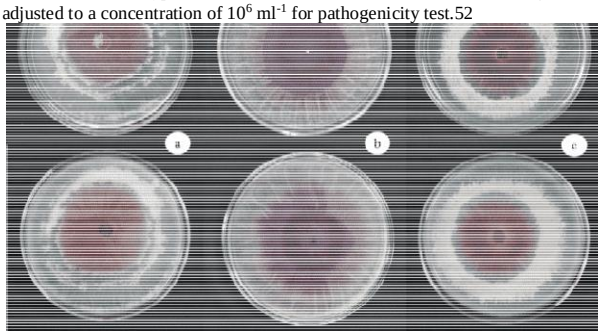

Figure1.*F.oxysporum*isolatesonPDAmedium.(a)AF01,(b)BF05, and(c)DF11.Firstline:Frontfrontview;secondline:reverse view.

Plantmaterial

Theplantsusedweremembersofthefabaceaefamily,namely*A.crassicarpa*,*A.auriculiformis*,*F.moluccana*,*A. pauciflorum*,*P.speciosa*,and*L.leucocephala*,whichwereonemonthold.Theseedlingswereobtainedfromtheforest CropsResearchInstitute,SouthSumatra.Seedlingsweretransferredinamixedmediumwithcocopeat(1:1)usinga plasticpotof10cmdiameterand10cmheight,andthenplacedinashadehouse.

Pathogenicitytest

Apathogenicitytestwascarriedoutusingrootdipmethod,inwhichtherootswerewashedunderrunningwaterand thenimmersedin250mlofconidiasuspension(10^6conidiaml^{-1})for15minutes.Thecontrolplantswereimmersedin steriledistilledwater,andtheseedlingsweretransplantedintoplasticpotsandplacedunderahouseshade.Eachisolate wasinoculatedon25plantswithfivereplicates(fiveplantsper-replicate).Then,diseaseseveritywascalculatedusingthe method~~designedby~~of Muslimetal.(2003a)and~~modifiedusing~~athe diseaseindex(DI)wasclassifiedintofollowing grades~~(DI)0-4~~,where0=nodisease/healthyseedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severe wilt,and4=deadseedling(ref).Furthermore,plantresponsesweregroupedas,R=resistant(DI=0),MR=moderately resistant/tolerance(DI=<1),MS=moderatelysusceptible(DI=1.0-2.0),S=susceptible(DI=2.1-3.0)andHS=highly susceptible(DI=3.1-4.0).Thedevelopmentofdiseasewasobserved1–21daysafterinoculation.

***Fusariumoxysporum*population**

Thepopulationof*F.oxysporum*intherootswascalculatedattheendoftheexperimentusingthemethodof(Muslim etal.2003b;Lietal.2008;Horinouchietal.2011)withmodificationstothesurfacesterilizationofsamples.Thenthe plantsweregroupedaccordingtoseverity(diseasescore)andwashedseparatelyunderrunningwatertoremovesoil residues.Afterthat,allplantsineachscoreweresurfacesterilisedusing1%sodiumhypochloritefor15minutes,then rinsedthreetimeswithdistilledwater.Thesamplesandwater(1:100w/v)werehomogenisedusingblenderat8000rpm for10minutes.Thentheywerefilteredusingtwolayersofsterilegauzeanddiluted10to1000times.Thesuspensionwas spreadonPeptonePCNBagar~~m~~Media(PPA/NashSnyderMedium)(LeslieandSummerrel,2006)intriplicate(fivePetri dishesperreplication)andincubatedindarkforsevendaysatroomtemperature.Thenumberofcolony-formingunits (CFU)of*F.oxysporum*wascalculatedonthebasisoffreshweightpergramofsampleandgroupedaccordingtothelevel ofdiseaseseverity~~level~~.~~recordedbasedonthediseaseseverity~~.

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RESULTSANDDISCUSSIONN

Pathogenicitytest

Theresultsshowedthatallthesixforestplantstestedhadsimilarreaction~~t~~othepathogen.Sevendaysafter inoculation,allthe plantsshowedtypicalsymptomsof*F.oxysporum*infection,i.e.yellowingofoldestleavesclosesttothe stembase,whichgraduallyprogresstoyoungershoots,severewilting,drying,fallingofleaves,andeventuallyplantdie. Anothersymptomthatappearedwassuddenwiltinganddeathofplantwithoutchangingtheleafcolour,whilecontrol plantsdidnotshowanysymptoms(Figure2,3,4,5,6&7).

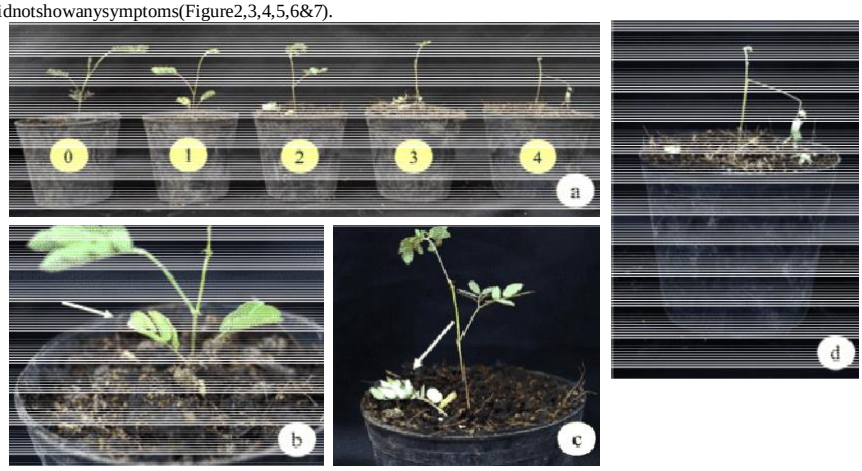

Figure2. DiseaseindexofA*cacia crassicarpa*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initialsymptoms: yellowingfromoldestleaves;(c)advancedsymptoms:fallingleaves;(d)deadplant.

Figure3. Diseaseindex on*Falcatariamoluccana*,(a)fromleft:healthy plant to100% wiltedleaves (score0−4); (b) initialsymptoms: yellowingfromoldestleaves;(c)advancedsymptoms:curved,dry,andfallingleaves;(d)deadplant.

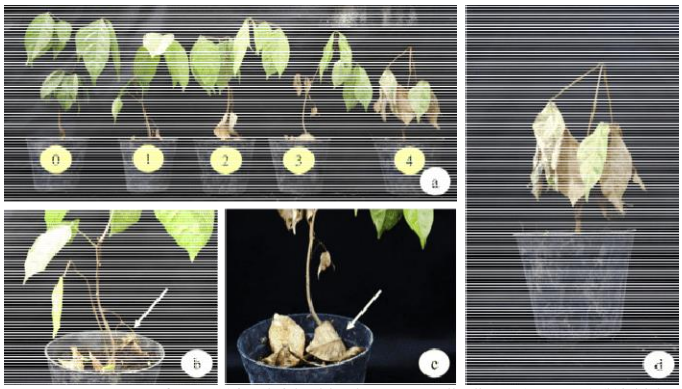

Figure4. Disease index on *Archidendron pauciflorum*, (a) from left: healthy plant to 100% wilted leaves (score 0−4), (b) initial symptoms: yellowing and dry from oldest leaves, (c) advanced symptoms: falling leaves, (d) dead plant.

Figure5. Disease index on *Leucaena leucocephala*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward.

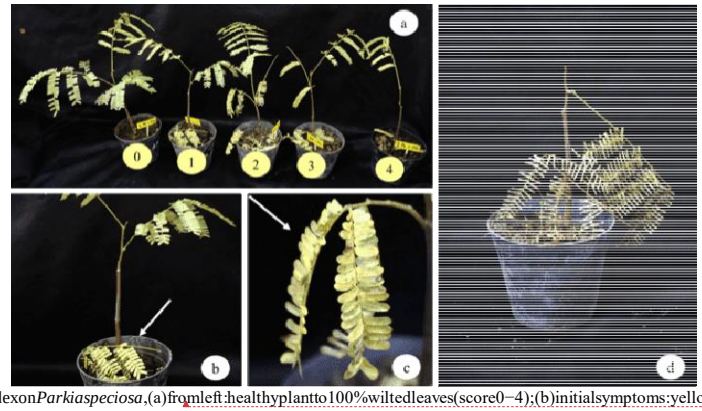

Figure6. Disease index on *Parkia speciosa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant.

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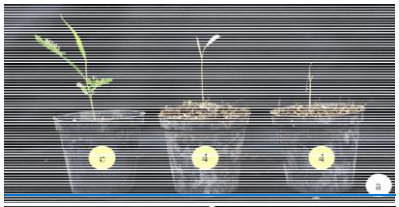

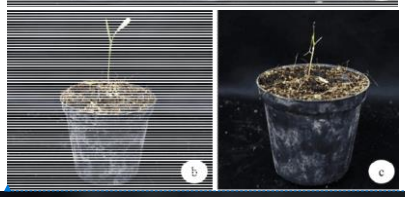

Figure 7. Disease index on*Acacia auriculiformis*,(a) from left: healthy plant to ~~100%~~ wilted ~~leaves~~ and dead plant (score 0 -4) and −-4));
(b and c) advanced symptoms: ……..

Diseaseseveritywassignificantlyhigherthancontrols. *A.crassicarpa*and *F.moluccana*weremostseverelyaffectedwithanaveragescoreof4.00and3.44,respectively~~andtheincidenceofwiltingwas100%~~.Onth eotherhand, *A.pauciflorum*, *L.leucocephala*,and *P.speciosa*wereshowedmoderatediseaseseverityi.e.1.96,1.68,and1.80,respectively,whereas *A.auriculiformis*hadthelowest(0.36)diseaseseverity(Table1)and(16%)incidence)(Table1).Basedonthediseasescore,hostplantswer eclassifiedintothreegroups:i)highlysusceptible(*A.crassicarpa*and *F.moluccana*),ii)moderatelysusceptible(*A.pauciflorum* *P.speciosa*,and *L.leucocephala*),andiii)moderateresistance/tolerance (*A. auriculiformis*) (Table 1).

.Resultexhibitedthattherewasnosignificantdifferencebetweenthediseaseseverityinthesamehostthathadbeeninoculated with different isolates.

Table1.~~Pathogenicityand~~~~disease~~Diseaseseverityandhostresponsestoof*Fusariumoxysporum*isolatedfrom *Acaciamangium*

Plantspecies	Isolates[a)]					
	AF01[b)]Response		[c)]BF05ResponseDF11Response			
Acaciacrassicarpa	4.00a	HS[c)]	3.48a	HS	3.96a	
	HS*Falcatariamoluccana*		3.44ab	HS	3.04a	HS
	2.80ab	S*Archidendronpauciflorum*		1.96bc	MS	
	1.88b	MS	1.40cd	MS*Leucaenaleucocephala*		
	1.52c	MS	1.56b	MS	1.68bc	
	MS*Parkiaspeciosa*		1.80c	MS	1.04bc	MS
	2.16bc	S				
Acaciaauriculiformis	0.36d	MR	0.40c	MR	0.60d	

MRValues followed by the same letter in each row are not significant.

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[a] DI0-4, where 0=no disease/healthy seedling, 1=yellow leaves, 2=yellow leaves and slightly wilted, 3=severe wilt, and 4=dead seedling.

[b)] *F. oxysporum* isolates.

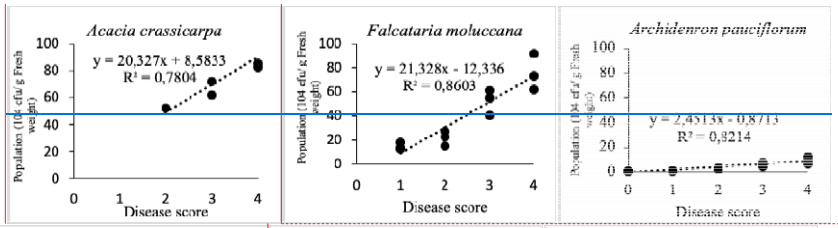
[c)] Host response ~~were~~ grouped as: R= resistant (DI = 0); MR= moderately resistant/tolerance (DI =<1); MS= moderately susceptible (DI=1.0-2.0); S= susceptible (DI=2.1-3.0); HS= highly susceptible (DI=3.1-4.0) (Bertettietal.2018)

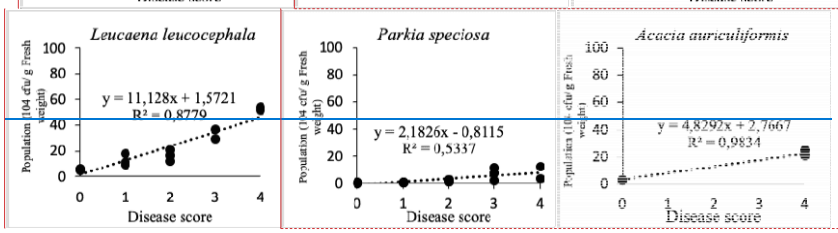
***Fusariumoxysporum* population**

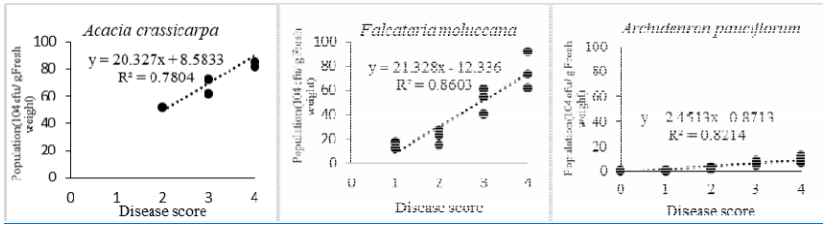
The total population of *F. oxysporum* on the roots was determined by calculating the CFU for each category of damage. The results showed that at a score of 4, *A. crassicarpa* and *F. moluccana* showed a significantly higher population (82.00–105.10 × 10^4 CFU g^{-1} fresh weight) than other plants. The lowest population was recorded in *P. speciosa* and *A. pauciflorum* (3.57–12.27×10^4 CFU g^{-1} fresh weight). This same pattern also occurred in DI 2 and 3, while no sample was recorded in *A. Auriculiformis* for DI 2. highest population was recorded in *F. moluccana* and *L. leucocephala*, while *A. crassicarpa* and *A. auriculiformis* had no sample for DI 1. In control plants (DI=0) ~~control plants (DI=0)~~, the population was significantly higher in *L. leucocephala* and *A. auriculiformis* and no sample was noted in *A. crassicarpa* and *F. moluccana* for ~~DI0~~ (Table 2 & Table 3).

The regression analysis results showed that all plants except *P. speciosa* had a linear relationship pattern between the increase in disease score and population. The pathogenic population on *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, as indicated by the magnitude of regression gradient coefficient (m=20.3–21.3). However, moderate increase was observed in *L. leucocephala* (m=11.2) (m=11.2) and very slow in *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* (m=2.2–4.8) (Figure 8).

Table 3 showed that isolates were different in *tef1-α*, but the population and DI patterns were similar for each test plant. The correlation between the population of pathogen (g^{-1} fresh weight) and the level of DI were described as follows: i) high pathogen populations with high DI (*A. crassicarpa* and *F. moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population with moderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).







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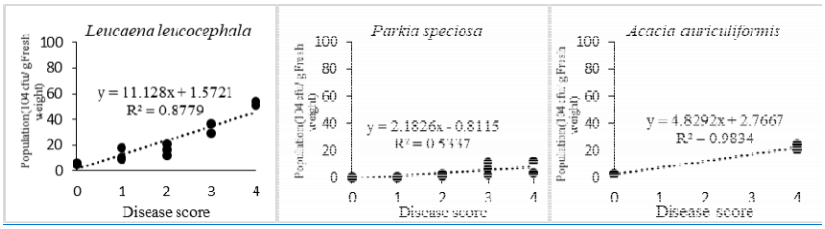

Figure8. Regressionanalysisofdiseasescorerateand*F.oxysporum*population

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Table 2. *Fusarium oxysporum* population onrootineachdiseaseindex

Plantspecies	Populationof*Fusariumoxysporum*(×10^4CFU/gfreshweight)[a]					Average[c]
	0[b]	1	2	3	4	
AF01[d]						
Acaciacrassicarpa	n.s	n.s	n.s	n.s	85.13a[e]	85.13
Falcatariamoluccana	n.s	17.,77a	22.77a	60.98a	91.87a	76.50
Archidendronpauciflorum	0.45b	1.10b	3.22b	8.15b	12.53cd	5.06
Leucaenaleucocephala	6.17a	18.10a	20.93a	n.s	51.67b	22.13
Parkia speciosa	0.32b	0.45b	2.58b	7.27b	3.57d	2.16
Acaciaauriculiformis	2.92a	n.s	n.s	n.s	24.53c	4.65
BF05						
Acaciacrassicarpa	n.s	n.s	51.80a	72.08a	105.10a	92.61
Falcatariamoluccana	n.s	13.22a	15.32b	40.33b	61.67b	43.85
Archidendronpauciflorum	0.47c	0.63b	1.73c	6.88c	9.90d	3.60
Leucaenaleucocephala	4.67a	9.02a	12.32b	29.32b	n.s	11.16
Parkia speciosa	0.48c	0.57b	1.27c	2.33d	n.s	0.87
Acaciaauriculiformis	2.55b	n.s	n.s	n.s	20.43c	3.98
DF11						
Acaciacrassicarpa	n.s	n.s	n.s	61.92a	82.00a	81.20
Falcatariamoluccana	n.s	12.50a	27.47a	54.93a	73.00a	47.93
Archidendronpauciflorum	0.35c	0.35b	3.37c	4.42c	6.92e	2.19
Leucaenaleucocephala	5.58a	11.17a	16.53b	36.63b	54.27b	19.69
Parkia speciosa	0.25c	0.48b	1.58c	11.97d	12.27d	5.79
Acaciaauriculiformis	2.83b	n.s	n.s	n.s	21.28c	5.05

n.s:No sample,cfu:colonyformingunit

[a] *F. oxysporum* populations calculatedat theendoftheexperiment (21daysafterinoculation).

[b] DI0-4;0=nodisease/healthyseedling;1=yellowleaves;2=yellowleavesandslightlywilted;3=severewilt;and4=dead seedling.

[c] Averageof*F.oxysporum*population(cfu/gfreshweight)=($P_0A+P_1B+P_2C+P_3D+P_4E$)/N;whereP0,P1,P2,P3,andP4=populationofpathogeninscore0,1,2,3,and4:A=numberofplantsonscore 0;B=numberofplants onscore1; C=numberofplantsonscore2;D=number ofplantsonscore3;E=numberof plants on score4; N= totalnumberofplants.

[d] *F.oxysporum*isolates

[e] Valuesfollowed bythesameletterineachrowarenot significant.

Table3. *Fusariumoxysporum*populationaverageanddiseasesindexofplant

Plantspecies	Populationaverage(×10^4CFU/gfresh weight)[a]			Diseaseindex[b]		
	AF01[c]	BF05	DF11	AF01	BF05	DF11
Acaciacrassicarpa	85.13	92.61	81.20	4.00	3.48	3.96
Falcatariamoluccana	76.50	43.85	47.93	3.44	3.04	2.80
Archidendronpauciflorum	5.06	3.60	2.19	1.96	1.88	1.40
Leucaenaleucocephala	22.13	11.16	19.69	1.52	1.56	1.68
Parkiaspeciosa	2.16	0.87	5.79	1.80	1.04	2.16
Acaciaauriculiformis	4.65	3.98	5.05	0.36	0.40	0.60

[a]Average of*F. oxysporum*population(cfu/gfreshweight)= ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N;wherePo,P1,P2,P3,andP4=population ofpathogenin score0,1,2,3,and4: A=numberofplantsonscore0;B=numberofplantsonscore1;C=numberofplantson score 2;D=numberofplantsonscore3;E=numberofplantsonscore;N=totalnumberofplants.

[b]DI0-4;0=nodisease/healthyseedling;1=yellowleaves;2=yellowleavesandslightlywilted;3=severewilt;and4= deadseedling.

[c]*F.oxysporum*isolates.

Discussion

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum*attackingcommercialnurseriesof *A.mMangium*inSouthSumatra(Solehaetal.2021).Therefore,theinvestigationofanew host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests alsoprovide information aboutplant species that have the potential to become alternative hostsor main hosts for the pathogen(Sampaio et al. 2021).

Theresultsindicatedthat *F.oxysporum*,whichcausesvascularwiltin*A.mangium*nursery,canalsoinfectfabaceaeplantswithvarioushostresponses..*A.crassicarpa*and *F.moluccana*werehighlysusceptible,while *A.pauciflorum*, *L.leucocephala*,and *P.speciosa*weremoderatelyvulnerable,and *A.auriculiformis*wasmoderatelyresistant.Pathogencausedwilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36)in *A. auriculiformis*, but ithadthepotentialtodamageplants. *F.oxysporum*abletoinfectplantsevenwithalowDI,causingthedeathofcultivars.Moreover,whenplantisgrownincontaminatedsoil,t hereisahighrisk kofdamagetocrops.Asimilarincidentwasreportedby Pastranaetal.(2017)-------,inwhich *F.oxysporum*fromblackberryalsocausedsuddendeathinstrawberries.;therefore,itbecomesathreatwhenplantedonadjacentland.Ano therexactcasewasreportedbyFarrow*oxysporum*causesdiseasesoninin Euphorbia(Bertettietal.2017). whencultivatedonthe sameland-------.

The results revealed that several types of plants belonging to the fabaceae family had great potential to become analternative hosts andeven main host for *F. oxysporum* whenplantedin the same field. Widespread of this pathogenmayallow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of newspeciesaffectedtheoccurrenceofnewoutbreaksbecausethepathogenicstrainsadaptedtothesoilandhadbecame virulent (Sampaioetal.2021;StukenbrockandMcDonald2008). Furthermore,nurseryactivitiesthatusecontaminated soilrepeatedlyalsotriggeredthepathogensproliferationandadaptationtootherplants.

Thepathogenpopulationin*A.crassicarpa*and*F.moluccana*grewveryrapidlywithincreasingdiseasescores,whilein *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* grew slowly. In this study, thepopulationof *F. oxysporum* onhighlysusceptible plants (*A. crassicarpa*and *F. moluccana*) was significantly higherthanotherplantsforeachdiseasescore.Thispatternwasiscommonwherethepopulationofthepathogenwasalsohigherwith the disease scores (Scott et al. (2014).This pattern was common where high disease scores were also found in the highpopulationdeBorbaetal.(2017)reportedthatsusceptiblelettucecultivarsshowedhigh*Fusarium*populationlevel and vulnerable black bean genotype showed a population level of 15.4 × 10^5 CFU g^{-1}(de Borba et al. 2017). (. In Tthe Thesecondpatternwasobservedon*L.leucocephala*,wherethepopulationofpathogenwasalsomoderatewithinamoderate amoderatediseasesscore.susceptiblehost(*L.leucocephala*). Thesimilarresultwasalsooccurredingarlicwithadiseas severityof44%dueto *Fusarium*spp.infection,whichshowedasignificantlymoderatehigher numberofpathogensonroots (Molinero-Ruiz et al. 2011).-----

Aspecialpatternoccurredon*A.pauciflorum*thatcouldhavebeenthatinwhich*F.oxysporum*causedby amoderat pathogeninfection,butthepathogenpopulationwaslow.Thismightbedueto theplantdefencemechanism. Scotteta (2014)-------reportedthatresistantpepperplantsalsosupportpathogendevelopmentinroots,evenwithout external symptoms.Similarphenomenawasreportedby Muslimetal.(2003a) -------whonotedthatsometomatoplantsare infected moderately (score 1-2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower thanother plants insamescore

Theinfectionandtotalpopulationon*Parkiaspeciosa*and*A.auriculiformis*waslower.Thisindicatedthatplanst Fangetal.(2012) plantsbelongedtotheresistantplantgroup. -------reportedthatwhenresistantstrawberryplantswere

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inoculatedwith *F.oxysporum*f.sp. *fragariae*,thecultivarformedabarrierwithaccumulatedphenoliccellsinthehypodermallayerthateffectivelylimitsthepathogencolonisationandpreventtheinvasionofrootvascultissue.Ifthetissuepenetrationbyhyphae waslimitedtothe~~epidermis~~epidermis,thenthepathogensdonotreachthevasculartissue.VanDenBergetal.(2007)~~.....~~reportedthatban anaclonestolerantto *F.oxysporum*f.sp. *cubense*correspondwiththis,withasignificantincreaseintheinductionofcellwall-associatedphenoliccompounds.Jiménez-Fernándezetal.(2013) ~~.....~~alsoreportedthat *Fusariumoxysporum*f.sp. *ciceris*race0remainedintheintercellularspaceofrootcortexandfailed to reach xylem in resistant chickpea cultivars.

Inthisstudy, *A.crassicarpa*and *F.moluccana* wereproventobeanalternativehostof *F. oxysporum*.Whereas *L.leucocephala*, *A.pauciflorum*, *P.speciosa*,and *A.auriculiformis*hadpotentialasalternativehosts.Manyplantsoffabaceae family was attacked byformae specialis *F. oxysporum*, suchas *Vigna angularis* (*F. oxysporum* f.sp. *adzukicola*),*Cicerarietinum*,*Cicer*spp.(*F.oxysporum* f.sp. *ciceris*), *Acacia*spp.(*F.oxysporum* f.sp. *koae*), *Lensculinaris*,*L.esculenta*(*F.oxysporum* f.sp. *lentis*), *Medicagosativa*(*F.oxysporum* f.sp. *medicaginis*), *Phaseolusvulgaris*, *P.coccineus*(*F.oxysporum* f.sp. *phaseoli*),*Pisumsativum*,*Cicerarietinum*(*F.oxysporum* f.sp. *pisi*)(Edel-HermannandLecomte2019)~~....~~.However,inthisstudy, *F.oxysporum*isolatedfrom*A.mangium*hasawidehostrangefromfabaceaefamily; therefore, it is not classified as formae specialis.

Inconclusion,*F.oxysporum*isolatedfrom*A.mangium*causesinfectioninseveraltypesofforestandindustrialplants. Sinceithasawidehostrange,itisnotclassifiedaspartoftheformaespecialisgroup.

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Answer Review 2

Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acaciamangium*

Abstract. *Fusarium oxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acaciamangium* in South Sumatra. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several forest and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef*1-α) sequences were tested for pathogenicity on different fabaceae family plants and the growth of population was also observed. The results showed that all the three isolates were able to infect all the tested plants with different reactions of wilt disease. The *Acaciacrassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendronpauciflorum*, *Leucaenaleucocephala*, and *Parkiaspeciosa* were moderately vulnerable and *Acaciaauriculiformis* was moderately resistant. The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the fabaceae family.

Keyword: *Acaciamangium*, fabaceae, *Fusarium oxysporum*, host range, seedling wilt

INTRODUCTION

Acaciamangium (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea, and Australia, and which, has also been found for a few decades in the humid tropical lowlands of Asia, South America, and Africa (Koutika and Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to various environmental conditions (Asi et al. 2017), it is widely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

Fusarium oxysporum is an important pathogenic fungus that causes wilt disease in different plants all over the world. Solehaet al. (2021) reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Ranaet al. 2017). Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds) (Joshi 2018). This fungus is also able to attack various plant habits such as trees (Zhang et al. 2013), herbaceous plants (Jacobs and Heerden 2012), and vines (Rooney-Latham and Blomquist 2011). Several types of forest plants that have reported lybeen attacked by *F. oxysporum* are *Pinus massoniana* (Luo and Yu 2020), *Tectonagrandis* (Borges et al. 2018), *Pseudotsugamenziesii* (Stewart et al. 2011), *Acacia mangium* (Widyastuti et al. 2013) and others.

Since *F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). According to Leslie and Summerell (2006) more than 100 formae species and race shave been identified and are widespread in the world.

Besides *A. mangium*, which is the main plant of industrial forestry in Indonesia, other plants, such as *Acacia crassicarpa*, *Acaciaauriculiformis*, *Parkiaspeciosa*, *Archidendronpauciflorum*, *Falcatariamoluccana*, and *Leucaena leucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can be come the main or alternative hosts for *F. oxysporum*, causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several industrial and local forest plants in Indonesia.

MATERIALSANDMETHOD

Fungalisolates

Threepathogenicisolatesof*F.oxysporum*(AF01,BF05,andDF11)wereselected,whichweredifferentiated accordingtotheir*tef*1-αsequence(Figure1).IsolateswereculturedonPDBliquidmedium(potatodextrosebroth)and incubatedat26-28°Conashaker(150rpm)foraboutfivedays.Thenthemyceliasuspensionwasfilteredusingtwo layersofsterilegauzetoseparatetheconidiaandhyphae.Theconidialconcentrationwasdeterminedusinga hemocytometer and then adjusted to a concentration of 10^6 ml^{-1} for pathogenicity test.

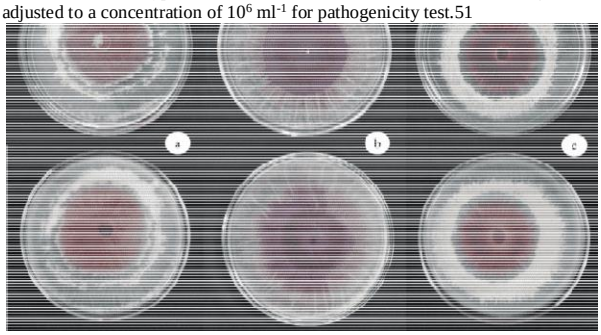

Figure1.*F.oxysporum*isolatesonPDAmedium.(a)AF01,(b)BF05,and(c)DF11.Firstline:frontview;secondline:reverseview.

Plantmaterial

Theplantsusedweremembersofthefabaceaefamily,namely*A.crassicarpa*,*A.auriculiformis*,*F.moluccana*,*A. pauciflorum*,*P.speciosa*,and*L.leucocephala*,whichwereonemonthold.Theseedlingswereobtainedfromthe Forest CropsResearchInstitute,SouthSumatra.Seedlingsweretransferredinamixedmediumwithcocopeat(1:1)usinga plasticpotof10cmdiameterand10cmheight,andthenplacedinashadehouse.

Pathogenicitytest

Apathogenicitytestwascarriedoutusingrootdipmethod,inwhichtherootswerewashedunderrunningwaterand thenimmersedin250mlofconidiasuspension(10^6conidiaml^{-1})for15minutes.Thecontrolplantswereimmersedin steriledistilledwater,andtheseedlingsweretransplantedintoplasticpotsandplacedunderahouseshade.Eachisolate wasinoculatedon25plantswithfivereplicates(fiveplantsper-replicate).Then,diseaseseverity was calculated using the methodofMuslimetal.(2003a)andthediseaseindex(DI)wasclassifiedintofollowinggrades,where0=no disease/healthyseedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severewilt,and4=deadseedling (Bertettietal.2018).Furthermore,plantresponsesweregroupedas,R=resistant(DI=0),MR=moderately resistant/tolerance(DI=<1),MS=moderatelysusceptible(DI=1.0–2.0),S=susceptible(DI=2.1–3.0)andHS=highly susceptible(DI=3.1–4.0).Thedevelopmentofdiseasewasobserved1–21daysafterinoculation.

Commented[AKG1]: Ifyouhavefollowedanyreferencefordisease index please mention the author name.

***Fusariumoxysporum*population**

Thepopulationof*F.oxysporum*intherootswascalculatedattheendoftheexperimentusingthemethodof(Muslim etal.2003b;Lietal.2008;Horinouchietal.2011)withmodificationstothesurfacesterilizationofsamples.Then the plantsweregroupedaccordingtoseverity(diseasescore)andwashedseparatelyunderrunningwatertoremovesoil residues.Afterthat,allplantsineachscoreweresurfacesterilisedusing1%sodiumhypochloritefor15minutes,then rinsedthreetimeswithdistilledwater.Thesamplesandwater(1:100w/v)werehomogenisedusingblenderat8000rpm for10minutes.Thentheywerefilteredusingtwolayersofsterilegauzeanddiluted10to1000times.Thesuspensionwas spreadonPeptonePCNBagarMedia(PPA/NashSnyderMedium)(LeslieandSummerell2006)intriplicate(fivePetri dishesperreplication)andincubatedindarkforsevendaysatroomtemperature.Thenumberofcolony-formingunits (CFU)of*F.oxysporum*wascalculatedonthebasisoffreshweightpergramofsampleandgroupedaccordingtothelevel ofdiseasesseverity.

RESULTS AND DISCUSSION

Pathogenicity test

The results showed that all the six forest plants tested had similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of *F. oxysporum* infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progress to young er shoots, severe wilting, drying, falling of leaves, and eventually plant die. Another symptom that appeared was sudden wilting and death of plant without changing the leaf colour, while control plants did not show any symptoms (Figures 2–7).

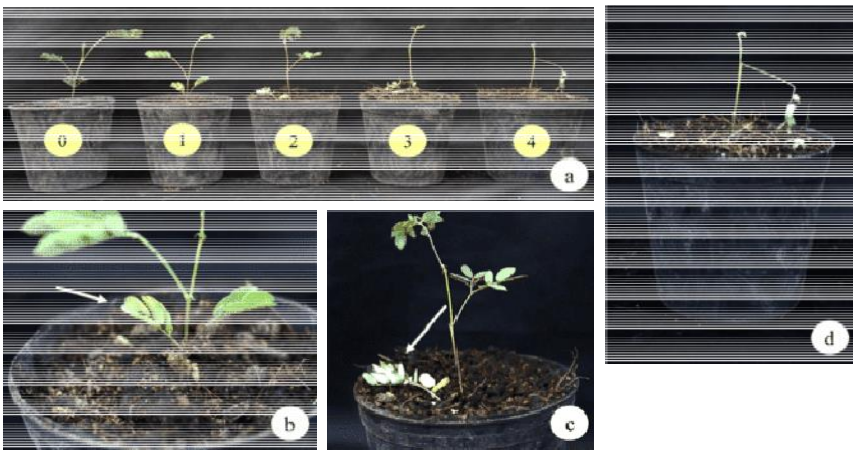

Figure 2. Disease index of *Acacia crassicarpa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

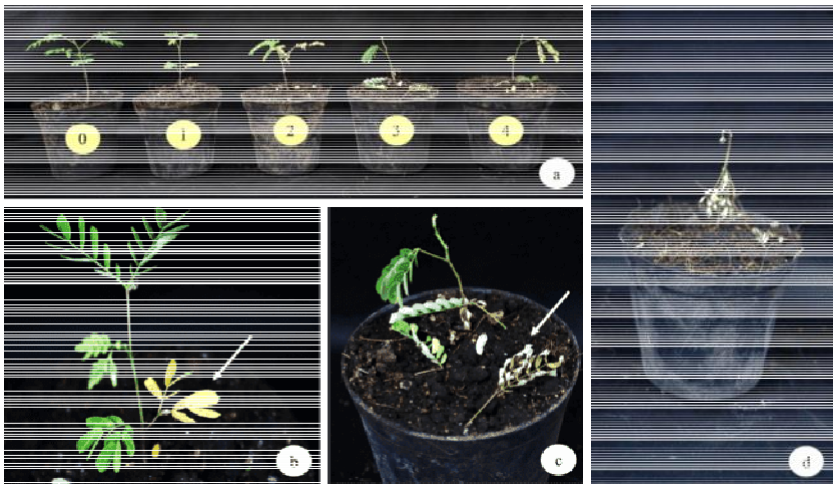

Figure 3. Disease index on *Falcataria moluccana*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved, dry, and falling leaves; (d) dead plant

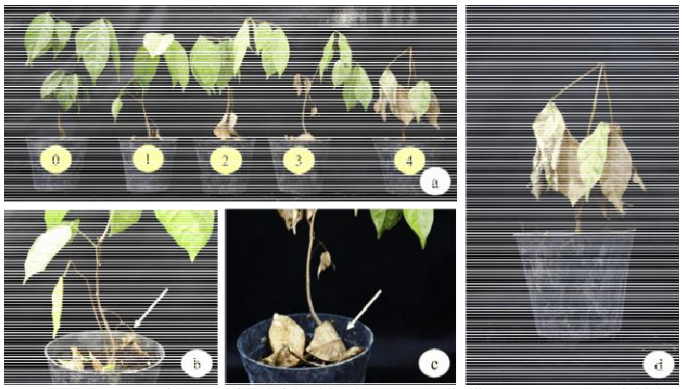

Figure4. Disease index on *Archidendron pauciflorum*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing a
dry from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

Figure5. Disease index on *Leucaena leucocephala*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing fr
oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward

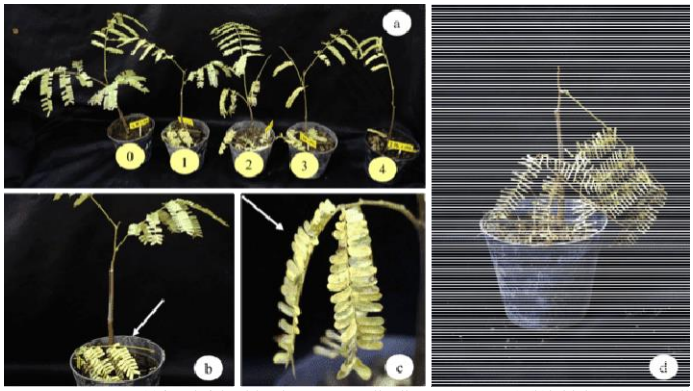

Figure6. Disease index on *Parkia speciosa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry fron
oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant

Figure7. Diseaseindexon*Acaciaauriculiformis*,fromleft:healthyplanttowiltedanddeadplant(score0–4)

Diseaseseveritywassignificantlyhigherthancontrols.*A.crassicarpa*and*F.moluccana*weremostseverelyaffected withanaveragescoreof4.00and3.44,respectively.Ontheotherhand,*A.pauciflorum*,*L.leucocephala*,and*P.speciosa* wereshowedmoderatediseaseseverityi.e.1.96,1.68,and1.80,respectively,whereas*A.auriculiformis*hadthelowest (0.36)diseaseseverity(Table1).Basedonthediseasescore,hostplantswereclassifiedintothreegroups:i)highly susceptible(*A.crassicarpa*and*F.moluccana*),ii)moderatelysusceptible(*A.pauciflorum*P.speciosa*,and*L. leucocephala*),andiii)moderateresistance/tolerance(*A.auriculiformis*).Resultexhibitedthattherewasnosignificant differencebetweenthediseaseseverityinthesamehostthathadbeeninoculatedwithdifferentisolates(Table1).

Table1. Diseaseseverityandhostresponsesto*Fusariumoxysporum*isolatedfrom*Acaciamangium*

Plantspecies	Isolates[a)]					
	AF01[b)]Response		[c)]BF05ResponseDF11Response			
Acaciacrassicarpa	4.00a	HS[c)]	3.48a	HS	3.96a	
	HS*Falcatariamoluccana*		3.44ab	HS	3.04a	HS
	2.80ab	S*Archidendronpauciflorum*		1.96bc	MS	
	1.88b	MS	1.40cd	MS*Leucaenaleucocephala*		
	1.52c	MS	1.56b	MS	1.68bc	
	MS*Parkiaspeciosa*		1.80c	MS	1.04bc	MS
	2.16bc	S*Acaciauriculiformis*		0.36d		
	MR0.40cMR0.60dMR					

Valuesfollowedbythesameletterineachrowarenotsignificant.
[a]DI0–4,where0=nodisease/healthyseedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severewilt,and 4 = dead seedling.
[b)]*F.oxysporum*isolates.
[c)] Host response grouped as: R = resistant (DI = 0); MR = moderately resistant/tolerance (DI = <1); MS = moderately susceptible (DI =1.0–2.0); S = susceptible (DI = 2.1–3.0); HS = highly susceptible (DI = 3.1–4.0) (Bertetti et al. 2018).

***Fusariumoxysporum*population**

Thetotalpopulationof *F.oxysporum*ontherootswasdeterminedbycalculatingtheCFUforeachcategoryofdamage.ForDI4,*A.crassicarpa*and*F.moluccana*s howedasignificantlyhigherpopulation(82.00–105.10×10^4CFUg^{-1}freshweight)thanotherplants.Thelowestpopulationwasrecordedin*P.speciosa*and*A.pauciflorum*(3.57–12.27×10^4 CFUg^{-1}freshweight).Thissamepatternalsooccurredin DI2and3,whilenosamplewasrecordedin*A.auriculiformis*forDI2and3.InDI1,thehigh estpopulationwasrecordedin *F.moluccana*and *L.leucocephala*,while *A.crassicarpa*and *A. auriculiformis* had no sample for DI 1. In ~~control plants~~inoculated plants with DI0~~ (DI=0)~~, the population wassignificantly higher in *L. leucocephala* and *A. auriculiformis* and no sample was noted in *A. crassicarpa* and *F. moluccana*(Table 2 &Table 3).

Theregressionanalysisresultsshowedthatallplantsexcept *P.speciosa*hadalinearrelationshippatternbetweentheincreaseindiseasescoreandpopulation.Thepathogenicpopulationon *A.crassicarpa*and *F.moluccana*grewrapidlyalong with theincrease in disease scores, as indicated by the magnitude ofregression gradient coefficient (m=20.3–21.3).However,moderateincreasewasobservedin *L.leucocephala*(m=11.2)(m=11.2)andveryslowin *A.pauciflorum*, *P.speciose,* and *A. auriculiformis* (m=2.2–4.8) (Figure 8).

Commented[AKG2]: Sstandsforsusceptiblebutinthetextyouhave classified *Falcataria moluccana* as highly susceptible host. Pleasecheckitagain

Commented [nd3R2]: We classified *Falcataria moluccana* ashighlysusceptiblehostbecausethisplantshowedhighlysusceptible(H S) responses againsttwo isolates (AF01andBF05)andonlyoneisolate (DF11) shows susceptible respose (S)

Commented[AKG4]: Sstandsforsusceptiblebutinthetextyouhave classified *Parkia speciosa* moderate susceptible host. Pleasecheck it again.

Commented [nd5R4]: We classified *Parkia speciosa* asmoderate susceptible host because this plant showed moderatesusceptible(MS)responsesagainsttwoisolates(AF01andBF0 5)and only one isolate (DF11) shows susceptible response (S).

Commented [AKG6]: Ihave asked you earlier that what do youmeanbynosamples.Doesitmeanthat*Fusaium*wasnotfoundin*A.Auri culiformis*Please clear it

Commented [nd7R6]: No sample means that there was notfoundoninoculated*Acaciauriculiformis*thatshoweddiseaseindex2 and 3. All inoculated plants showed only disease index 0 anddisease index 4. In this experiment we observed population of*Fusariumoxysporum*basedondiseaseindexoftheinoculatedplant.

Commented[AKG8]: Againthesamequestionthatlhaveaskedyouear lier thatwhatdoyou meanby nosamples. Does it meanthat*Fusaium* was not found in *A. Auriculiformis*and *A. crassicarpa*. Pleaseclearit.

Commented [nd9R8]: No sample means that there was notfoundoninoculated*A.auriculiformis*and*A.crassicarpa*thatshoweddi sease index 1. All inoculated *A. auriculiformis* showed onlydiseases index 0 and 4. While *A. crassicarpa* showed only diseaseindex4(AF01);diseaseindex2,3,and4(BF05);anddiseaseindex3 and 4 (DF11). In this experiment we observed population of*Fusarium oxysporum* based on disease index of the inoculated plant.

Commented[nd10R8]: Answerline136-137(confusingline):*Fusariumoxysporum* did not foundon controluninoculatedplants.DI 0 means that disease index for inoculated *L. leucocephala*,*Leucaena leucocephala*, *Parkia speciosa* and *A. auriculiformis.*
While, *A. crassicarpa* and *F. moluccana* did not produce DI 0 (It ismean no sample for *A. crassicarpa* and *F. moluccana*), because allplantsshowedsymptoms(Table2).Wehavechange"controlplants"to be "inoculated plants"

Table 3showedthatisolateswere different in *tef1-α*, butthepopulation and DIpatternsweresimilarforeach testplant. Thecorrelationbetween the population of pathogen (g^{-1} fresh weight) and the level of DI were described as follows: i) high pathogen populations withhigh DI (*A. crassicarpa* and*F. moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population withmoderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).

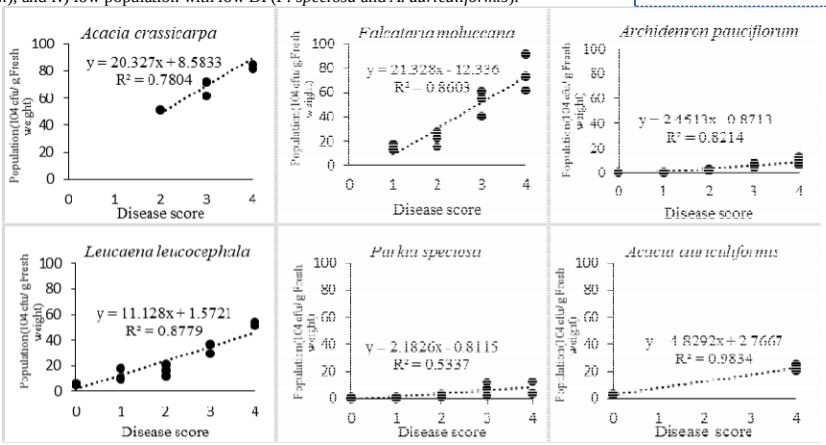

Figure8. Regressionanalysisofdiseasescorerateand*F.oxysporum*population

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Table 2. *Fusarium oxysporum* population onrootineachdiseaseindex

Plantspecies	Populationof*Fusariumoxysporum*(×10^4CFU/gfreshweight)[a]					Average[c]
	0[b]	1	2	3	4	
AF01[d]						
Acaciacrassicarpa	n.s	n.s	n.s	n.s	85.13a[e]	85.13
Falcatariamoluccana	n.s	17.77a	22.77a	60.98a	91.87a	76.50
Archidendronpauciflorum	0.45b	1.10b	3.22b	8.15b	12.53cd	5.06
Leucaenaleucocephala	6.17a	18.10a	20.93a	n.s	51.67b	22.13
Parkia speciosa	0.32b	0.45b	2.58b	7.27b	3.57d	2.16
Acaciaauriculiformis	2.92a	n.s	n.s	n.s	24.53c	4.65
BF05						
Acaciacrassicarpa	n.s	n.s	51.80a	72.08a	105.10a	92.61
Falcatariamoluccana	n.s	13.22a	15.32b	40.33b	61.67b	43.85
Archidendronpauciflorum	0.47c	0.63b	1.73c	6.88c	9.90d	3.60
Leucaenaleucocephala	4.67a	9.02a	12.32b	29.32b	n.s	11.16
Parkia speciosa	0.48c	0.57b	1.27c	2.33d	n.s	0.87
Acaciaauriculiformis	2.55b	n.s	n.s	n.s	20.43c	3.98
DF11						
Acaciacrassicarpa	n.s	n.s	n.s	61.92a	82.00a	81.20
Falcatariamoluccana	n.s	12.50a	27.47a	54.93a	73.00a	47.93
Archidendronpauciflorum	0.35c	0.35b	3.37c	4.42c	6.92e	2.19
Leucaenaleucocephala	5.58a	11.17a	16.53b	36.63b	54.27b	19.69
Parkia speciosa	0.25c	0.48b	1.58c	11.97d	12.27d	5.79
Acaciaauriculiformis	2.83b	n.s	n.s	n.s	21.28c	5.05

n.s:No sample,cfu:colonyformingunit

[a] *F. oxysporum* populationcalculatedat theendoftheexperiment(21daysafterinoculation).

[b] DI0−4;0=nodisease/healthyseedling;1=yellowleaves;2=yellowleavesandslightlywilted;3=severewilt;and4=dead seedling.

[c] Averageof*F.oxysporum*population(cfu/gfreshweight)=($P_0A+P_1B+P_2C+P_3D+P_4E$)/N;wherePO,P1,P2,P3,andP4=populationofpathogeninscore0,1,2,3,and4:A=numberofplantsonscore 0;B=numberofplants onscore1; C=numberofplantsonscore2;D=number ofplantsonscore3;E=numberof plants on score4; N= totalnumberofplants.

[d] *F.oxysporum*isolates

[e] Valuesfollowed bythesameletterineachrowarenot significant.

Table3. *Fusarium oxysporum* population average and disease index of plant

Plant species	Population average ($\times 10^4$ CFU/g fresh weight)[a]			Disease index[b]		
	AF01[c]	BF05	DF11	AF01	BF05	DF11
Acacia crassicarpa	85.13	92.61	81.20	4.00	3.48	3.96
Falcataria moluccana	76.50	43.85	47.93	3.44	3.04	2.80
Archidendron pauciflorum	5.06	3.60	2.19	1.96	1.88	1.40
Leucaena leucocephala	22.13	11.16	19.69	1.52	1.56	1.68
Parkia speciosa	2.16	0.87	5.79	1.80	1.04	2.16
Acacia auriculiformis	4.65	3.98	5.05	0.36	0.40	0.60

[a] Average of *F. oxysporum* population (cfu/g fresh weight) = $(P_0A+P_1B+P_2C+P_3D+P_4E)/N$; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.

[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] *F. oxysporum* isolates.

Discussion

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Solehaet al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information about plant species that have the potential to become alternative host or main hosts for the pathogen (Sampaio et al. 2021).

The results indicated that *F. oxysporum*, which causes vascular wilt in *A. mangium* nursery, can also infect fabaceae plants with various host responses. *A. crassicarpa* and *F. moluccana* were highly susceptible, while *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen caused wilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in *A. auriculiformis*, but it had the potential to damage plants. *F. oxysporum* able to infect plants even with a low DI, causing the death of cultivars. Moreover, when a plant is grown in contaminated soil, there is a high risk of damage to crops. A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from black berry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot diseases in *Euphorbia* (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative host and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrence of new outbreaks because the pathogenic strains adapted to the soil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the pathogens proliferation and adaptation to other plants.

The pathogen population in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing disease scores, while in *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* grew slowly. In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern is common where the population of pathogen is also higher with disease scores (Scott et al. 2014). de Borba et al. (2017) reported that susceptible lettuce cultivars showed high *Fusarium* population level and vulnerable black bean genotypes showed a population level of 15.4×10^5 CFU^{g-1}. The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate disease sess score. The similar result was also occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

A special pattern occurred on *A. pauciflorum* in which *F. oxysporum* caused a moderate infection, but the pathogen population was low. This might be due to the plant defence mechanism. Scott et al. (2014) reported that resistant pepper plants also support pathogen development in roots, even without external symptoms. Similar phenomena was reported by Muslimet al. (2003a) who noted that some tomato plants are infected moderately (score 1–2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower than other plants in same score.

The infection and total population on *Parkia speciosa* and *A. auriculiformis* was lower. This indicated that plants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with *F. oxysporum* f. sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonisation and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited to the epidermis, then the pathogens do not reach the vascular tissue. Van Den Berge et al. (2007) reported that banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond with this, with a significant increase in the

inductionofcellwall-associatedphenoliccompounds.Jiménez-Fernándezetal.(2013)alsoreportedthat*Fusarium oxysporum*f.sp.*ciceris*race0remainedintheintercellularspaceofrootcortexandfailedtoreachxyleminresistant chickpeacultivars.

Inthisstudy,*A.crassicarpa*and*F.moluccana*wereproventobeanalternativehostof*F.oxysporum*.Whereas*L. leucocephala*,*A.pauciflorum*,*P.speciosa*,and*A.auriculiformis*hadpotentialasalternativehosts.Manyplantsof fabaceaefamilywasattackedbyformaespecialis*F.oxysporum*,suchas*Vignaangularis*(*F.oxysporum*f.sp.*adzukicola*), *Cicerarietinum*,*Cicer*spp.(*F.oxysporum*f.sp.*ciceris*),*Acacia*spp.(*F.oxysporum*f.sp.*koae*),*Lensculinaris*,*L. esculenta*(*F.oxysporum*f.sp.*lentis*),*Medicagosativa*(*F.oxysporum*f.sp.*medicaginis*),*Phaseolusvulgaris*,*P. coccineus*(*F.oxysporum*f.sp.*phaseoli*),*Pisumsativum*,*Cicerarietinum*(*F.oxysporum*f.sp.*pisi*)(Edel-Hermannand Lecomte2019).However,inthisstudy,*F.oxysporum*isolatedfrom*A.mangium*hasawidehostrangefromfabaceae family;therefore,itisnotclassifiedasformaespecialis.

Inconclusion,*F.oxysporum*isolatedfrom*A.mangium*causesinfectioninseveraltypesofforestandindustrialplants. Sinceithasawidehostrange,itisnotclassifiedaspartoftheformaespecialisgroup.

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Thankyouverymuchforyouremailregardingreviewer'srevisionofourmanuscript.

We have revised and edited the text and the title of figure 8 of our manuscript (Line: 146 and line 150 (figure8title))asReviewer'srevision.Encloseourfinalrevisedmanuscriptentitled"HostRangeStudies of *Fusarium oxysporum*, Causal agent of Seedling Wilt Disease of*Acacia mangium*" by SolehaSoleha, Ahmad Muslim, Suwandi Suwandi, Sabaruddin Kadir, Rahmat Pratama.

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Here we re-submit our revised manuscript for publication in Biodiversitas Journal of BiologicalDiversity.

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AhmadMuslim,Ph.D Associate Professor
FacultyofAgriculture,SriwijayaUniversity
Jl.Palembang-PrabumulihKm.32,Indralaya,Palembang,Indonesia E-mail: a_muslim@unsri.ac.id
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Review3

Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acaciamangium*

Abstract. *Fusarium oxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acaciamangium* in South Sumatra. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several forest and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef*1-α) sequences were tested for pathogenicity on different fabaceae family plants and the growth of population was also observed. The results showed that all the three isolates were able to infect all the tested plants with different reactions of wilt disease. The *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauciflorum*, *Leucaena leucocephala*, and *Parkia speciosa* were moderately vulnerable and *Acacia auriculiformis* was moderately resistant. The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the fabaceae family.

Keyword: *Acaciamangium*, fabaceae, *Fusarium oxysporum*, host range, seedling wilt

INTRODUCTION

Acaciamangium (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea, and Australia, and which, has also been found for a few decades in the humid tropical lowlands of Asia, South America, and Africa (Koutika and Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to various environmental conditions (Asi et al. 2017), it is widely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

Fusarium oxysporum is an important pathogenic fungus that causes wilt disease in different plants all over the world. Solehaet al. (2021) reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Ranaet al. 2017). Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds) (Joshi 2018). This fungus is also able to attack various plant habits such as trees (Zhang et al. 2013), herbaceous plants (Jacobs and Heerden 2012), and vines (Rooney-Latham and Blomquist 2011). Several types of forest plants that have reported lybeen attacked by *F. oxysporum* are *Pinus massoniana* (Luo and Yu 2020), *Tectona grandis* (Borges et al. 2018), *Pseudotsuga menziesii* (Stewart et al. 2011), *Acacia mangium* (Widyastuti et al. 2013) and others.

Since *F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). According to Leslie and Summerell (2006) more than 100 formae species and race have been identified and are widespread in the world.

Besides *A. mangium*, which is the main plant of industrial forestry in Indonesia, other plants, such as *Acacia crassicarpa*, *Acacia auriculiformis*, *Parkia speciosa*, *Archidendron pauciflorum*, *Falcataria moluccana*, and *Leucaena leucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can become the main or alternative hosts for *F. oxysporum*, causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several industrial and local forest plants in Indonesia.

MATERIALSANDMETHOD

Fungalisolates

Threepathogenicisolatesof*F.oxysporum*(AF01,BF05,andDF11)wereselected,whichweredifferentiated accordingtotheir*tef*1-αsequence(Figure1).IsolateswereculturedonPDBliquidmedium(potatodextrosebroth)and incubatedat26-28°Conashaker(150rpm)foraboutfivedays.Thenthemyceliasuspensionwasfilteredusingtwo layersofsterilegauzetoseparatetheconidiaandhyphae.Theconidialconcentrationwasdeterminedusinga hemocytometer and then adjusted to a concentration of 10^6 ml^{-1} for pathogenicity test.

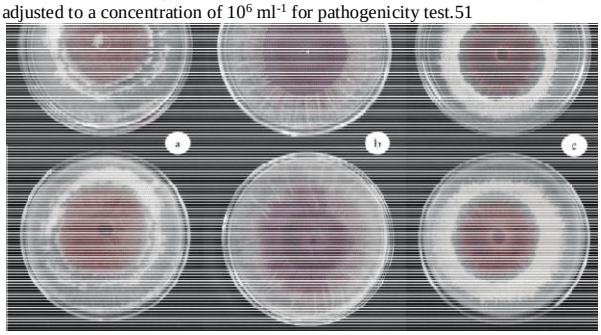

Figure1.*F.oxysporum*isolatesonPDAmedium.(a)AF01,(b)BF05,and(c)DF11.Firstline:frontview;secondline:reverseview.

Plantmaterial

Theplantsusedweremembersofthefabaceaefamily,namely*A.crassicarpa*,*A.auriculiformis*,*F.moluccana*,*A. pauciflorum*,*P.speciosa*,and*L.leucocephala*,whichwereonemonthold.TheseedlingswereobtainedfromtheForest CropsResearchInstitute,SouthSumatra.Seedlingsweretransferredinamixedmediumwithcocopeat(1:1)usinga plasticpotof10cmdiameterand10cmheight,andthenplacedinashadehouse.

Pathogenicitytest

Apathogenicitytestwascarriedoutusingrootdipmethod,inwhichtherootswerewashedunderrunningwaterand thenimmersedin250mlofconidiasuspension(10^6conidiaml^{-1})for15minutes.Thecontrolplantswereimmersedin steriledistilledwater,andtheseedlingsweretransplantedintoplasticpotsandplacedunderahouseshade.Eachisolate wasinoculatedon25plantswithfivereplicates(fiveplantsper-replicate).Then,diseaseseveritywascalculatedusingthe methodofMuslimetal.(2003a)andthediseaseindex(DI)wasclassifiedintofollowinggrades,where0=no disease/healthyseedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severewilt,and4=deadseedling (Bertettietal.2018).Furthermore,plantresponsesweregroupedas,R=resistant(DI=0),MR=moderately resistant/tolerance(DI=≤1),MS=moderatelysusceptible(DI=1.0–2.0),S=susceptible(DI=2.1–3.0)andHS=highly susceptible(DI=3.1–4.0).Thedevelopmentofdiseasewasobserved1–21daysafterinoculation.

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***Fusariumoxysporum*population**

ThepopulationofF.oxysporumintherootswascalculatedattheendoftheexperimentusingthemethodof(Muslim etal.2003b;Lietal.2008;Horinouchietal.2011)withmodificationstothesurfacesterilizationofsamples.Thenthe plantsweregroupedaccordingtoseverity(diseasescore)andwashedseparatelyunderrunningwatertoremovesoil residues.Afterthat,allplantsineachscoreweresurfacesterilisedusing1%sodiumhypochloritefor15minutes,then rinsedthreetimeswithdistilledwater.Thesamplesandwater(1:100w/v)werehomogenisedusingblenderat8000rpm for10minutes.Thentheywerefilteredusingtwolayersofsterilegauzeanddiluted10to1000times.Thesuspensionwas spreadonPeptonePCNBagarMedia(PPA/NashSnyderMedium)(LeslieandSummerell2006)intriplicate(fivePetri dishesperreplication)andincubatedindarkforsevendaysatroomtemperature.Thenumberofcolony-formingunits (CFU)of*F.oxysporum*wascalculatedonthebasisoffreshweightpergramofsampleandgroupedaccordingtothelevel ofdiseasesseverity.

RESULTS AND DISCUSSION

Pathogenicity test

The results showed that all the six forest plants tested had similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of *F. oxysporum* infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progress to younger shoots, severe wilting, drying, falling of leaves, and eventually plant die. Another symptom that appeared was sudden wilting and death of plant without changing the leaf colour, while control plants did not show any symptoms (Figures 2–7).

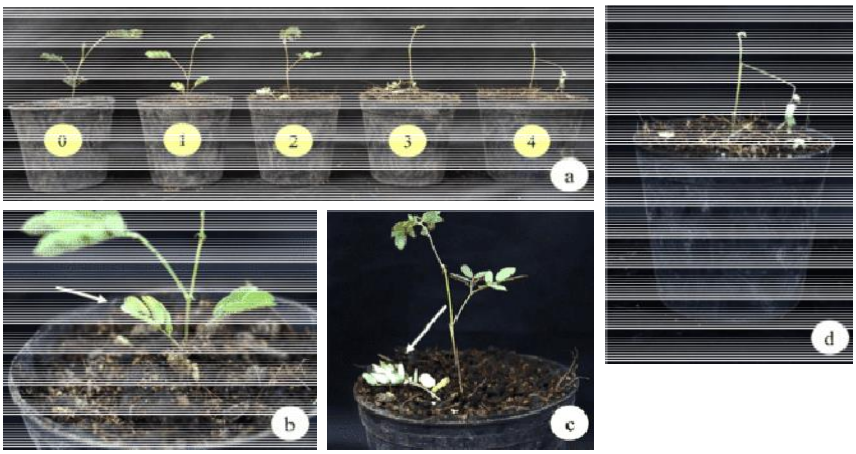

Figure 2. Disease index of *Acacia crassicarpa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

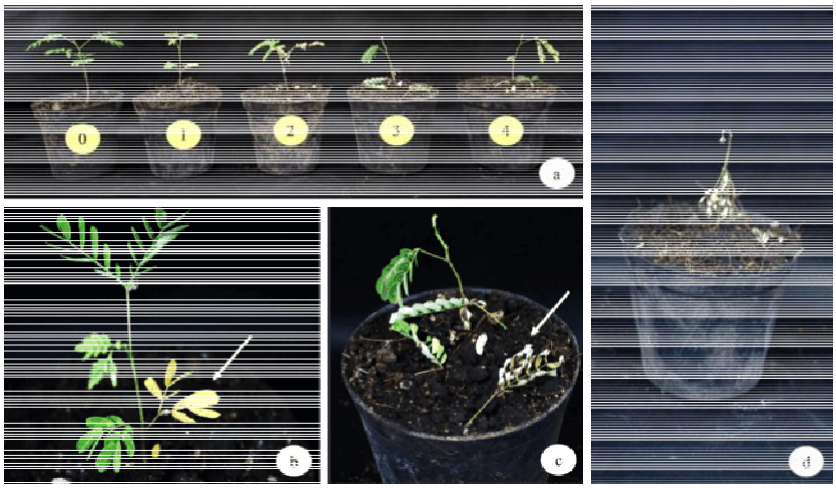

Figure 3. Disease index on *Falcataria moluccana*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved, dry, and falling leaves; (d) dead plant

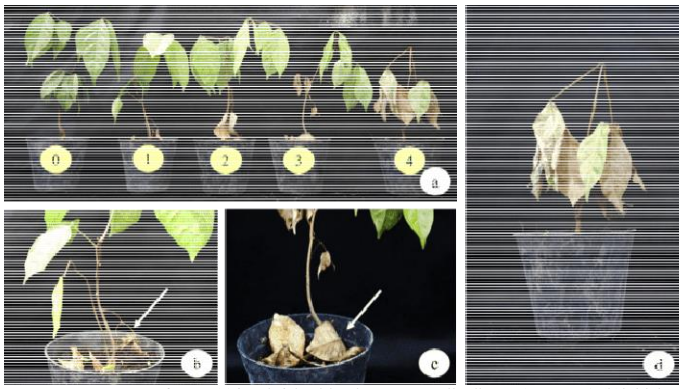

Figure4. Disease index on *Archidendron pauciflorum*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing a
dry from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

Figure5. Disease index on *Leucaena leucocephala*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing fr
oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward

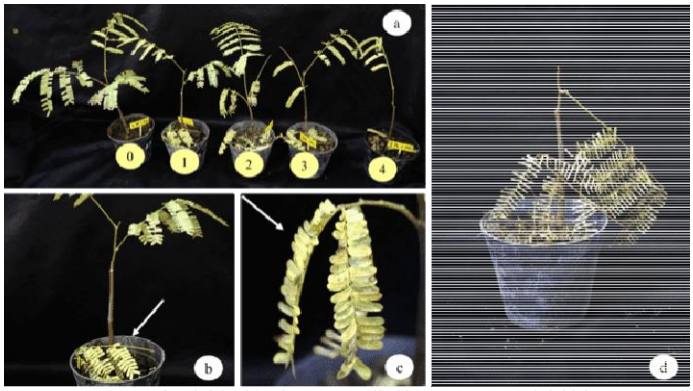

Figure6. Disease index on *Parkia speciosa*, (a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing and dry fron
oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant

Figure7. Diseaseindexon*Acaciaauriculiformis*,fromleft:healthyplanttowiltedanddeadplant(score0–4)

Diseaseseveritywassignificantlyhigherthancontrols.*A.crassicarpa*and*F.moluccana*weremostseverelyaffected withanaveragescoreof4.00and3.44,respectively.Ontheotherhand,*A.pauciflorum*,*L.leucocephala*,and*P.speciosa* wereshowedmoderatediseaseseverityi.e.1.96,1.68,and1.80,respectively,whereas*A.auriculiformis*hadthelowest (0.36)diseaseseverity(Table1).Basedonthediseasescore,hostplantswereclassifiedintothreegroups:i)highly susceptible(*A.crassicarpa*and*F.moluccana*),ii)moderatelysusceptible(*A.pauciflorumP.speciosa*,and*L. leucocephala*),andiii)moderateresistance/tolerance(*A.auriculiformis*).Resultexhibitedthattherewasnosignificant differencebetweenthediseaseseverityinthesamehostthathadbeeninoculatedwithdifferentisolates(Table1).

Table1. DiseaseseverityandhostresponsestoFusariumoxysporumisolatedfromAcaciamangium

Plantspecies	Isolates[a)]					
	AF01[b)]Response		[c)]BF05ResponseDF11Response			
Acaciacrassicarpa	4.00a	HS[c)]	3.48a	HS	3.96a	
	HS*Falcatariamoluccana*		3.44ab	HS	3.04a	HS
	2.80ab	S*Archidendronpauciflorum*		1.96bc	MS	
	1.88b	MS	1.40cd	MS*Leucaenaleucocephala*		
	1.52c	MS	1.56b	MS	1.68bc	
	MS*Parkiaspeciosa*		1.80c	MS	1.04bc	MS
	2.16bc	S*Acaciauriculiformis*		0.36d		
	MR0.40cMR0.60dMR					

Valuesfollowedbythesameletterineachrowarenotsignificant.
[a]DI0–4,where0=nodisease/healthyseedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severewilt,and 4 = dead seedling.
[b)]*F.oxysporum*isolates.
[c)] Host response grouped as: R = resistant (DI = 0); MR = moderately resistant/tolerance (DI = <1); MS = moderately susceptible (DI =1.0–2.0); S = susceptible (DI = 2.1–3.0); HS = highly susceptible (DI = 3.1–4.0) (Bertetti et al. 2018).

***Fusariumoxysporum*population**

Thetotalpopulationof *F.oxysporum*ontherootswasdeterminedbycalculatingtheCFUforeachcategoryofdamage.ForDI4,*A.crassicarpa*and*F.moluccana*s howedasignificantlyhigherpopulation(82.00–105.10×10^4CFUg^{-1}freshweight)thanotherplants.Thelowestpopulationwasrecordedin*P.speciosa*and*A.pauciflorum*(3.57–12.27×10^4 CFUg^{-1}freshweight).Thissamepatternalsooccurredin DI2and3,whilenosamplewasrecordedin*A.auriculiformis*forDI2and3.InDI1,thehigh estpopulationwasrecordedin *F.moluccana*and *L.leucocephala*,while *A.crassicarpa*and *A. auriculiformis* had no sample for DI 1. In ~~control plants~~inoculated plants with DI0~~ (DI=0)~~, the population wassignificantly higher in *L. leucocephala* and *A. auriculiformis* and no sample was noted in *A. crassicarpa* and *F. moluccana*(Table 2 &Table 3).

Theregressionanalysisresultsshowedthatallplantsexcept *P.speciosa*hadalinearrelationshippatternbetweentheincreaseindiseasescoreandpopulation.Thepathogenicpopulationon *A.crassicarpa*and *F.moluccana*grewrapidlyalong with theincrease in disease scores, as indicated by the magnitude ofregression gradient coefficient (m=20.3–21.3).However,moderateincreasewasobservedin *L.leucocephala*(m=11.2)(m=11.2)andveryslowin *A.pauciflorum*, *P.speciose*, and *A. auriculiformis* (m=2.2–4.8) (Figure 8).

Commented[AKG2]: Sstandsforsusceptiblebutinthetextyouhave classified *Falcataria moluccana* as highly susceptible host. Pleasecheckitagain

Commented [nd3R2]: We classified *Falcataria moluccana* ashighlysusceptiblehostbecausethisplantshowedhighlysusceptible(H S) responses againsttwo isolates (AF01andBF05)andonlyoneisolate (DF11) shows susceptible respose (S)

Commented[AKG4]: Sstandsforsusceptiblebutinthetextyouhave classified *Parkia speciosa* moderate susceptible host. Pleasecheck it again.

Commented [nd5R4]: We classified *Parkia speciosa* asmoderate susceptible host because this plant showed moderatesusceptible(MS)responsesagainsttwoisolates(AF01andBF0 5)and only one isolate (DF11) shows susceptible response (S).

Commented [AKG6]: Ihave asked you earlier that what do youmeanbynosamples.Doesitmeanthat*Fusaium*wasnotfoundin*A.Auri culiformis*Please clear it

Commented [nd7R6]: No sample means that there was notfoundoninoculated*Acaciauriculiformis*thatshoweddiseaseindex2 and 3. All inoculated plants showed only disease index 0 anddisease index 4. In this experiment we observed population of*Fusariumoxysporum*basedondiseaseindexoftheinoculatedplant.

Commented[AKG8]: Againthesamequestionthatlhaveaskedyouear lier thatwhatdoyou meanby nosamples. Does it meanthat*Fusaium* was not found in *A. Auriculiformis*and *A. crassicarpa*. Pleaseclearit.

Commented [nd9R8]: No sample means that there was notfoundoninoculated*A.auriculiformis*and*A.crassicarpa*thatshoweddi sease index 1. All inoculated *A. auriculiformis* showed onlydiseases index 0 and 4. While *A. crassicarpa* showed only diseaseindex4(AF01);diseaseindex2,3,and4(BF05);anddiseaseindex3 and 4 (DF11). In this experiment we observed population of*Fusarium oxysporum* based on disease index of the inoculated plant.

Commented[nd10R8]: Answerline136-137(confusingline):*Fusariumoxysporum* did not foundon controluninoculatedplants.DI 0 means that disease index for inoculated *L. leucocephala*,*Leucaena leucocephala*, *Parkia speciosa* and *A. auriculiformis.*
While, *A. crassicarpa* and *F. moluccana* did not produce DI 0 (It ismean no sample for *A. crassicarpa* and *F. moluccana*), because allplantsshowedsymptoms(Table2).Wehavechange"controlplants"to be "inoculated plants"

Table 3showedthatisolateswere different in *tef1-α*, butthepopulation and DIpatternsweresimilarforeach testplant. Thecorrelationbetween the population of pathogen (g^{-1} fresh weight) and the level of DI were described as follows: i) high pathogen populations withhigh DI (*A. crassicarpa* and*F. moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population withmoderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).

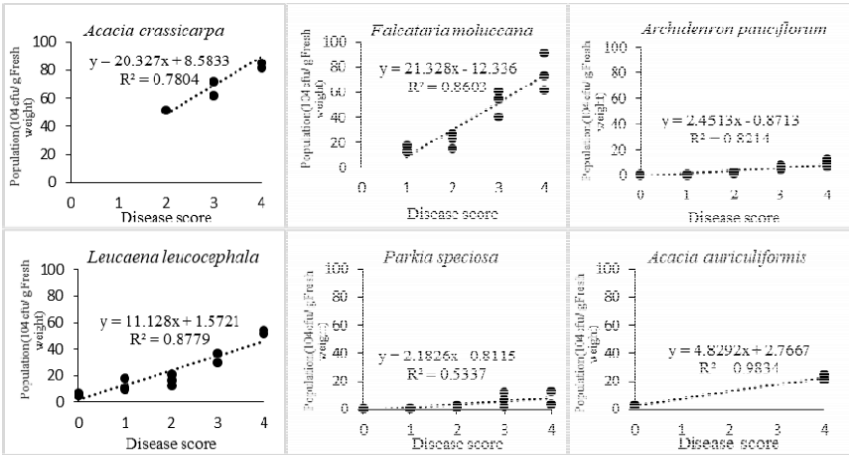

Figure8. Regressionanalysisofdiseasescorerateand*F.oxysporum*population

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Table 2. *Fusarium oxysporum* population onrootineachdiseaseindex

Plantspecies	Populationof*Fusariumoxysporum*(×10^4CFU/gfreshweight)[a]					Average[c]
	0[b]	1	2	3	4	
AF01[d]						
Acaciacrassicarpa	n.s	n.s	n.s	n.s	85.13a[e]	85.13
Falcatariamoluccana	n.s	17.77a	22.77a	60.98a	91.87a	76.50
Archidendronpauciflorum	0.45b	1.10b	3.22b	8.15b	12.53cd	5.06
Leucaenaleucocephala	6.17a	18.10a	20.93a	n.s	51.67b	22.13
Parkia speciosa	0.32b	0.45b	2.58b	7.27b	3.57d	2.16
Acaciaauriculiformis	2.92a	n.s	n.s	n.s	24.53c	4.65
BF05						
Acaciacrassicarpa	n.s	n.s	51.80a	72.08a	105.10a	92.61
Falcatariamoluccana	n.s	13.22a	15.32b	40.33b	61.67b	43.85
Archidendronpauciflorum	0.47c	0.63b	1.73c	6.88c	9.90d	3.60
Leucaenaleucocephala	4.67a	9.02a	12.32b	29.32b	n.s	11.16
Parkia speciosa	0.48c	0.57b	1.27c	2.33d	n.s	0.87
Acaciaauriculiformis	2.55b	n.s	n.s	n.s	20.43c	3.98
DF11						
Acaciacrassicarpa	n.s	n.s	n.s	61.92a	82.00a	81.20
Falcatariamoluccana	n.s	12.50a	27.47a	54.93a	73.00a	47.93
Archidendronpauciflorum	0.35c	0.35b	3.37c	4.42c	6.92e	2.19
Leucaenaleucocephala	5.58a	11.17a	16.53b	36.63b	54.27b	19.69
Parkia speciosa	0.25c	0.48b	1.58c	11.97d	12.27d	5.79
Acaciaauriculiformis	2.83b	n.s	n.s	n.s	21.28c	5.05

n.s:No sample,cfu:colonyformingunit

[a] *F. oxysporum* populationcalculatedat theendoftheexperiment(21daysafterinoculation).

[b] DI0−4;0=nodisease/healthyseedling;1=yellowleaves;2=yellowleavesandslightlywilted;3=severewilt;and4=deadseedling.

[c] Averageof*F.oxysporum*population(cfu/gfreshweight)=($P_0A+P_1B+P_2C+P_3D+P_4E$)/N;wherePO,P1,P2,P3,andP4=populationofpathogeninscore0,1,2,3,and4:A=numberofplantsonscore 0;B=numberofplants onscore1; C=numberofplantsonscore2;D=number ofplantsonscore3;E=numberof plants on score4; N= totalnumberofplants.

[d] *F.oxysporum*isolates

[e] Valuesfollowed bythesameletterineachrowarenot significant.

Table3. *Fusarium oxysporum* population average and disease sindex of plant

Plant species	Population average ($\times 10^4$ CFU/g fresh weight)[a]			Disease index[b]		
	AF01[c]	BF05	DF11	AF01	BF05	DF11
Acacia crassicarpa	85.13	92.61	81.20	4.00	3.48	3.96
Falcataria moluccana	76.50	43.85	47.93	3.44	3.04	2.80
Archidendron pauciflorum	5.06	3.60	2.19	1.96	1.88	1.40
Leucaena leucocephala	22.13	11.16	19.69	1.52	1.56	1.68
Parkia speciosa	2.16	0.87	5.79	1.80	1.04	2.16
Acacia auriculiformis	4.65	3.98	5.05	0.36	0.40	0.60

[a] Average of *F. oxysporum* population (cfu/g fresh weight) = $(P_0A+P_1B+P_2C+P_3D+P_4E)/N$; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.

[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] *F. oxysporum* isolates.

Discussion

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Solehaet al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information about plant species that have the potential to become alternative host or main hosts for the pathogen (Sampaio et al. 2021).

The results indicated that *F. oxysporum*, which causes vascular wilt in *A. mangium* nursery, can also infect fabaceae plants with various host responses. *A. crassicarpa* and *F. moluccana* were highly susceptible, while *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen caused wilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in *A. auriculiformis*, but it had the potential to damage plants. *F. oxysporum* able to infect plants even with a low DI, causing the death of cultivars. Moreover, when a plant is grown in contaminated soil, there is a high risk of damage to crops. A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from black berry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot diseases in *Euphorbia* (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative host and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrence of new outbreaks because the pathogenic strains adapted to the soil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the pathogens proliferation and adaptation to other plants.

The pathogen population in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing diseases scores, while in *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* grew slowly. In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern is common where the population of pathogen is also higher with disease scores (Scott et al. 2014). de Borba et al. (2017) reported that susceptible lettuce cultivars showed high *Fusarium* population level and vulnerable black bean genotypes showed a population level of 15.4×10^5 CFU^{g-1}. The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate diseases score. The similar result was also occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

A special pattern occurred on *A. pauciflorum* in which *F. oxysporum* caused a moderate infection, but the pathogen population was low. This might be due to the plant defence mechanism. Scott et al. (2014) reported that resistant pepper plants also support pathogen development in roots, even without external symptoms. Similar phenomena was reported by Muslimet al. (2003a) who noted that some tomato plants are infected moderately (score 1–2) by *F. oxysporum* f. sp. *lycopersici*, but the population was lower than other plants in same score.

The infection and total population on *Parkia speciosa* and *A. auriculiformis* was lower. This indicated that plants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with *F. oxysporum* f. sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonisation and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited to the epidermis, then the pathogens do not reach the vascular tissue. Van Den Berge et al. (2007) reported that banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond with this, with a significant increase in the

inductionofcellwall-associatedphenoliccompounds.Jiménez-Fernándezetal.(2013)alsoreportedthat*Fusarium oxysporum*f.sp.*ciceris*race0remainedintheintercellularspaceofrootcortexandfailedtoreachxylemin resistant chickpeacultivars.

Inthisstudy,*A.crassicarpa*and*F.moluccana*wereproventobeanalternativehostof*F.oxysporum*.Whereas*L. leucocephala*,*A.pauciflorum*,*P.speciosa*,and*A.auriculiformis*hadpotentialasalternativehosts.Manyplantsof fabaceaefamilywasattackedbyformaespecialis*F.oxysporum*,suchas*Vignaangularis*(*F.oxysporum*f.sp.*adzukicola*), *Cicerarietinum*,*Cicer*spp.(*F.oxysporum*f.sp.*ciceris*),*Acacia*spp.(*F.oxysporum*f.sp.*koae*),*Lensculinaris*,*L. esculenta*(*F.oxysporum*f.sp.*lentis*),*Medicagosativa*(*F.oxysporum*f.sp.*medicaginis*),*Phaseolusvulgaris*,*P. coccineus*(*F.oxysporum*f.sp.*phaseoli*),*Pisumsativum*,*Cicerarietinum*(*F.oxysporum*f.sp.*pisi*)(Edel-Hermannand Lecomte2019).However,inthisstudy,*F.oxysporum*isolatedfrom*A.mangium*hasawidehostrangefromfabaceae family;therefore,itisnotclassifiedasformaespecialis.

Inconclusion,*F.oxysporum*isolatedfrom*A.mangium*causesinfectioninseveraltypesofforestandindustrialplants. Sinceithasawidehostrange,itisnotclassifiedaspartoftheformaespecialis group.

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Answer Review 3

Host range studies of *Fusarium oxysporum*, causal agent of seedling wilt disease of *Acacia mangium*

Abstract. *Fusarium oxysporum* is a serious pathogen that causes severe wilt disease in commercial nurseries of *Acacia mangium* in South Sumatra. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several forest and industrial plants. Three isolates of *F. oxysporum* with different translation elongation factor (*tef* 1-α) sequences were tested for pathogenicity on different fabaceae family plants and the growth of population was also observed. The results showed that all the three isolates were able to infect all the tested plants with different reactions of wilt disease. The *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauciflorum*, *Leucaena leucocephala*, and *Parkia speciosa* were moderately vulnerable and *Acacia auriculiformis* was moderately resistant. The pathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while in *L. leucocephala* it was moderate, and slow in *A. pauciflorum*, *P. speciosa* and *A. auriculiformis* plants. In conclusion, *F. oxysporum* pathogen, which was isolated from *A. mangium*, has a wide range of hosts in the fabaceae family.

Keyword: *Acacia mangium*, fabaceae, *Fusarium oxysporum*, host range, seedling wilt

INTRODUCTION

Acacia mangium (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea, and Australia, and which, has also been found for a few decades in the humid tropical lowlands of Asia, South America, and Africa (Koutikaand Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to various environmental conditions (Asifetal.2017), it is widely used for agroforestry, forestry, and restoration of degraded land (Koutikaand Richardson 2019).

Fusarium oxysporum is an important pathogenic fungus that causes wilt disease in different plants all over the world. Solehaetal.(2021) reported that it was identified as the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra. The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatovetal.2012; Koyyappurathetal.2016; Ranaetal.2017). Furthermore, it attacks almost every type of plant, from cultivated to forest and wild (e.g. weeds) (Joshi 2018). This fungus is also able to attack various plant habits such as trees (Zhangetal.2013), herbaceous plants (Jacobs and Heerden 2012), and vines (Rooney-Latham and Blomquist 2011). Several types of forest plants that have reportedly been attacked by *F. oxysporum* are *Pinus massoniana* (Luo and Yu 2020), *Tectona grandis* (Borgesetal.2018), *Pseudotsuga menziesii* (Stewartetal.2011), *Acacia mangium* (Widyastutietal.2013) and others.

Since *F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardtetal.2019; Tayloretal.2019). According to Leslie and Summerell (2006) more than 100 formae species and race have been identified and are widespread in the world.

Besides *A. mangium*, which is the main plant of industrial forestry in Indonesia, other plants, such as *Acacia crassicarpa*, *Acacia auriculiformis*, *Parkia speciosa*, *Archidendron pauciflorum*, *Falcataria moluccana*, and *Leucaena leucocephala* are also important and have high economic value. Considering that they belong to the same family (Fabaceae), they can become the main or alternative hosts for *F. oxysporum*, causative agent of wilt disease. This study aimed to investigate the host range of *F. oxysporum* as a nursery wilt pathogen in *A. mangium* and several industrial and local forest plants in Indonesia.

MATERIALSANDMETHOD

Fungalisolates

Threepathogenicisolatesof*F.oxysporum*(AF01,BF05,andDF11)wereselected,whichweredifferentiated accordingtotheir*tef*1-αsequence(Figure1).IsolateswereculturedonPDBliquidmedium(potatodextrosebroth)and incubatedat26-28°Conashaker(150rpm)foraboutfivedays.Thenthemyceliasuspensionwasfilteredusingtwo layersofsterilegauzetoseparatetheconidiaandhyphae.Theconidialconcentrationwasdeterminedusinga hemocytometerandthenadjustedtoaconcentrationof10^6ml^{-1}forpathogenicitytest.

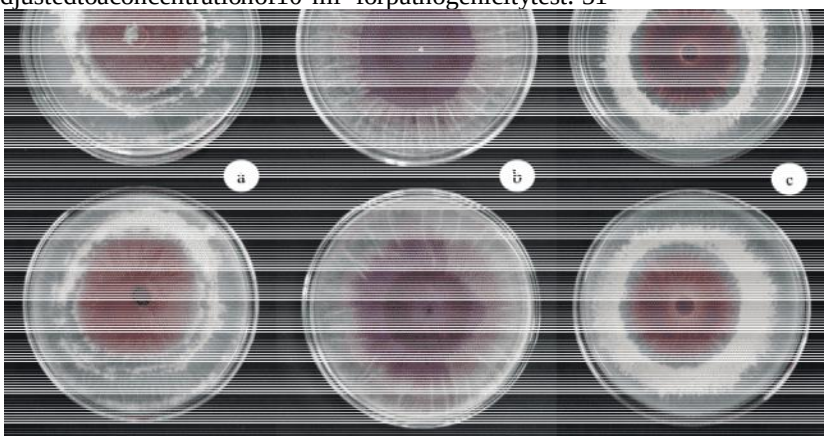

Figure1.*F.oxysporum*isolatesonPDAmedium.(a)AF01,(b)BF05,and(c)DF11.Firstline:frontview;secondline:reverseview.

Plantmaterial

Theplantsusedweremembersofthefabaceaefamily,namely*A.crassicarpa,A.auriculiformis,F.moluccana,A. pauciflorum,P.speciosa*,and*L.leucocephala*,whichwereonemonthold.TheseedlingswereobtainedfromtheForest CropsResearchInstitute,SouthSumatra.Seedlingsweretransferredinamixedmediumwithcocopeat(1:1)usinga plasticpotof10cmdiameterand10cmheight,andthenplacedinashadehouse.

Pathogenicitytest

Apathogenicitytestwascarriedoutusingrootdipmethod,inwhichtherootswerewashedunderrunningwaterand thenimmersedin250mlofconidiasuspension(10^6conidiaml^{-1})for15minutes.Thecontrolplantswereimmersedin steriledistilledwater,andtheseedlingsweretransplantedintoplasticpotsandplacedunderahouseshade.Eachisolate wasinoculatedon25 plantswithfivereplicates (five plantsper-replicate).Then,disease severitywascalculated usingthe methodofMuslimetal.(2003a)andthediseaseindex (DI)wasclassifiedintofollowinggrades,where0=no disease/healthyseedling,1=yellowleaves,2=yellowleavesandslightlywilted,3=severewilt,and4=deadseedling (Bertettietal.2018).Furthermore,plantresponsesweregroupedas,R=resistant(DI=0),MR=moderately resistant/tolerance(DI=<1),MS=moderatelysusceptible(DI=1.0–2.0),S=susceptible(DI=2.1–3.0)andHS=highly susceptible(DI=3.1–4.0).Thedevelopmentofdiseasewasobserved1–21daysafterinoculation.

***Fusariumoxysporum*population**

Thepopulationof*F.oxysporum*intherootswascalculatedattheendoftheexperiment usingthemethodof(Muslim etal.2003b;Lietal.2008;Horinouchietal.2011)withmodificationstothesurfacesterilizationofsamples.Thenthe plantsweregroupedaccordingtoseverity(diseasescore)andwashedseparatelyunderrunningwatertoremovesoil residues.Afterthat,allplantsineachscoreweresurfacesterilisedusing1%sodiumhypochloritefor15minutes,then rinsedthree timeswithdistilledwater.The samplesandwater(1:100w/v)were homogenisedusingblenderat8000rpm for10minutes.Thentheywerefilteredusingtwolayersofsterilegauzeanddiluted10to1000times.Thesuspensionwas spreadonPeptonePCNBagarMedia(PPA/NashSnyderMedium)(LeslieandSummerell2006)intriplicate(fivePetri dishesperreplication)andincubatedindarkforsevendaysatroomtemperature.Thenumberofcolony-formingunits (CFU)of*F.oxysporum* wascalculatedon thebasisoffreshweightpergramofsampleandgrouped accordingtothelevel ofdiseasesseverity.

RESULTSANDDISCUSSION

Pathogenicitytest

Theresultsshowedthatallthesixforestplantstestedhadsimilarreactiontothepathogen.Sevendaysafter inoculation,alltheplantsshowedtypicalsymptomsof*F.oxysporum*infection,i.e.yellowingofoldestleavesclosestto the stembase,whichgraduallyprogress toyoungershoots,severewilting,drying,fallingof leaves, andeventuallyplantdie. Anothersymptomthatappearedwassuddenwiltinganddeathofplantwithoutchangingtheleafcolour,whilecontrol plantsdidnotshowanysymptoms(Figures2–7).

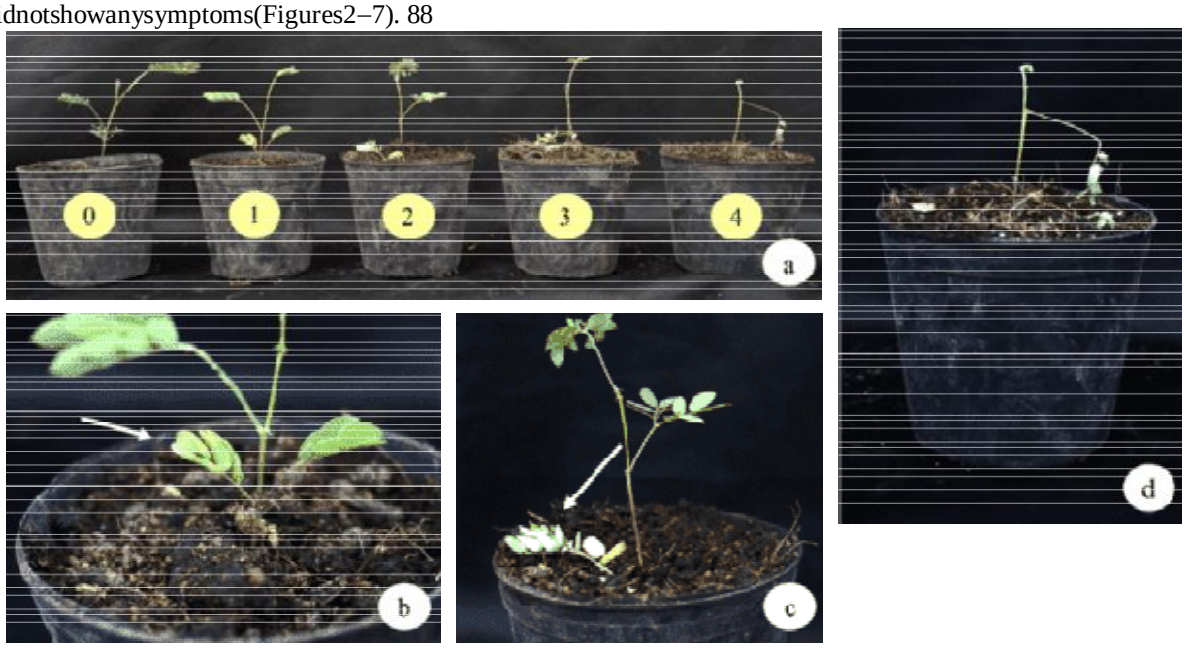

Figure2. DiseaseindexofA*cacia crassicarpa*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initialsymptoms: yellowingfromoldestleaves;(c)advancedsymptoms:fallingleaves;(d)deadplant

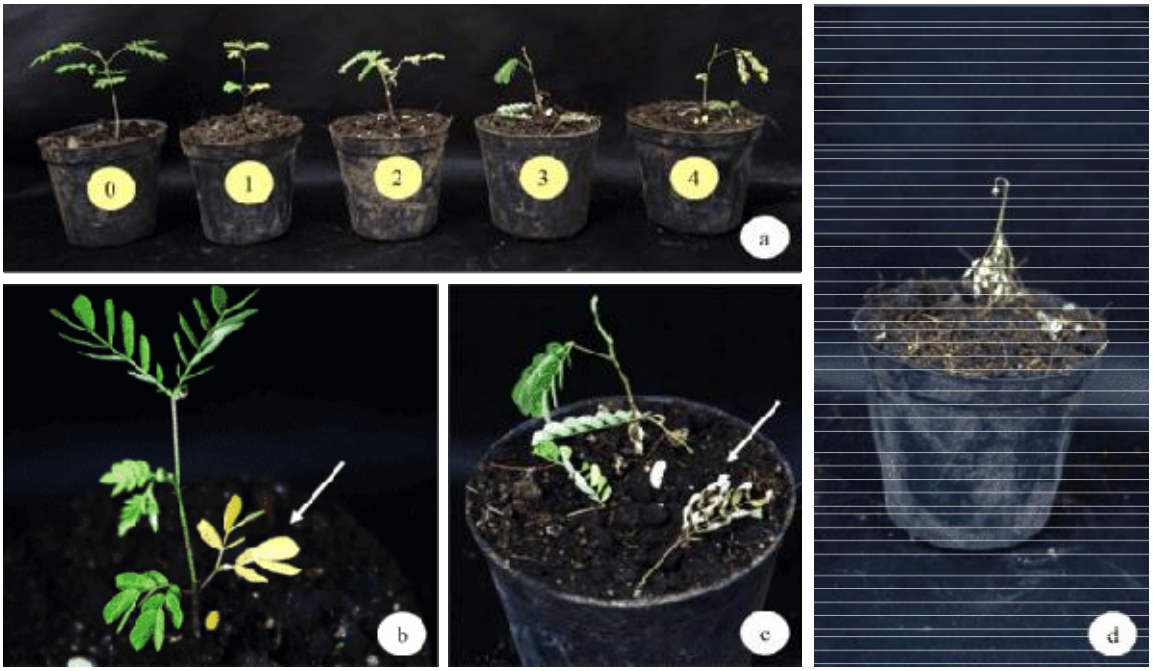

Figure3. Disease index on*Falcatariamoluccana*,(a) fromleft:healthyplantto100% wiltedleaves(score 0−4);(b)initial symptoms: yellowingfromoldestleaves;(c)advancedsymptoms:curved,dry,andfallingleaves;(d)deadplant

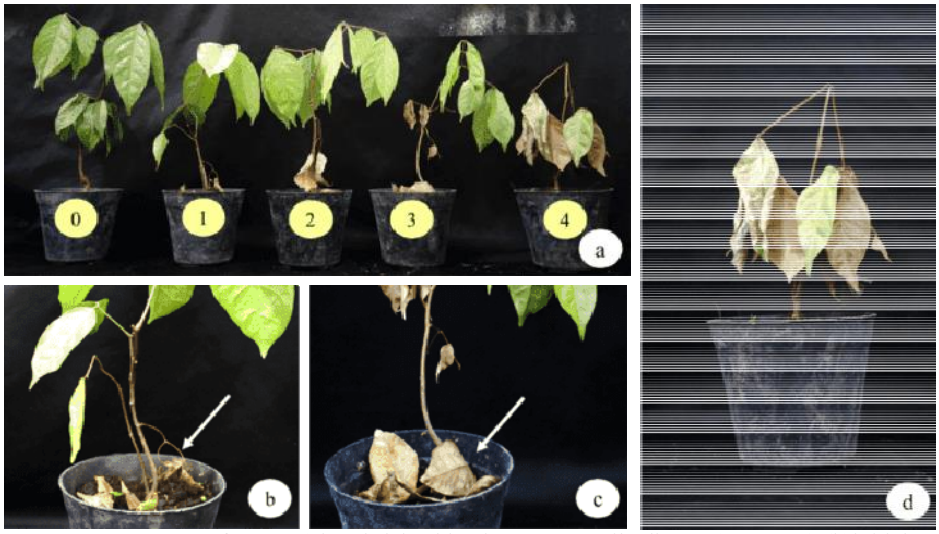

Figure4. Diseaseindexon*Archidendronpauciflorum*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

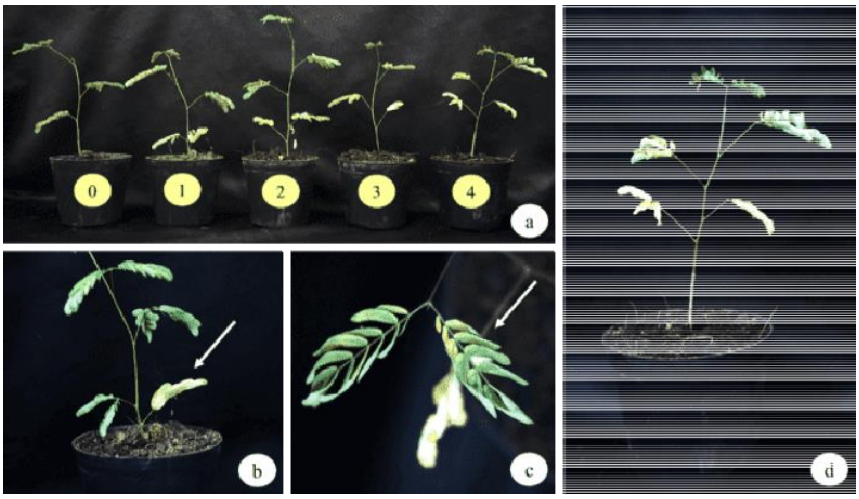

Figure5. Diseaseindexon*Leucaenaleucocephala*,(a)fromleft:healthyplant to100%wilted leaves(score0−4);(b)initialsymptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward

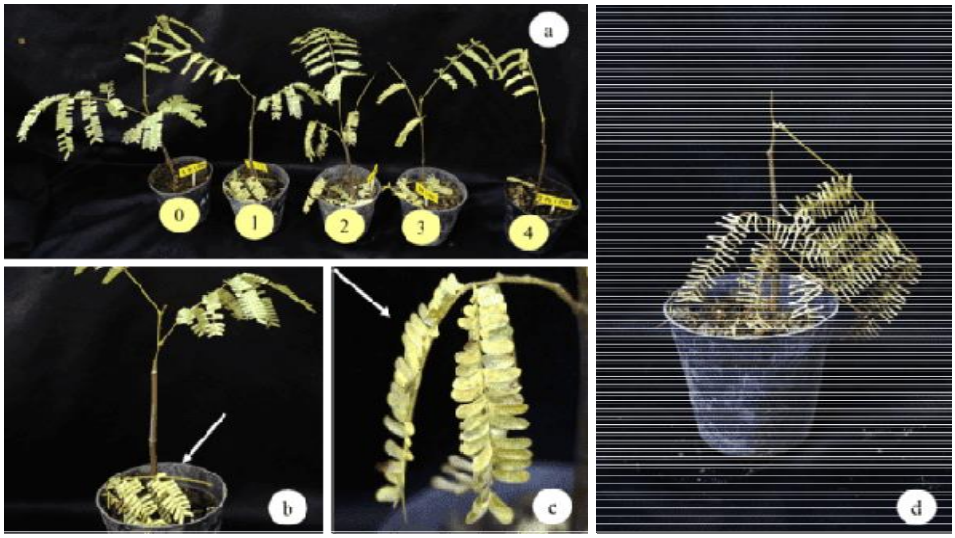

Figure6. Diseaseindexon*Parkiaspeciosa*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initialsymptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant

Figure7. Diseaseindexon*Acaciaauriculiformis*,fromleft:healthyplanttowiltedanddeadplant(score0–4)

Diseaseseveritywassignificantlyhigherthancontrols.*A.crassicarpa*and*F.moluccana*weremostseverelyaffected withanaveragescoreof4.00and3.44,respectively.Ontheotherhand,*A.pauciflorum*,*L.leucocephala*,and*P.speciosa* wereshowedmoderatediseaseseverityi.e.1.96,1.68,and1.80,respectively,whereas*A.auriculiformis*hadthelowest (0.36)diseaseseverity(Table1).Basedonthediseasescore,hostplantswereclassifiedintothreegroups:i)highly susceptible(*A.crassicarpa*and*F.moluccana*),ii)moderatelysusceptible(*A.pauciflorumP.speciosa*,and*L. leucocephala*),andiii)moderateresistance/tolerance(*A.auriculiformis*).Resultexhibitedthattherewasnosignificant differencebetweenthediseaseseverityinthesamehostthathadbeeninoculatedwithdifferentisolates(Table1).

Table1. Diseaseseverityandhostresponsesto*Fusariumoxysporum*isolatedfrom*Acaciamangium*

Plantspecies	Isolates[a)]					
	AF01[b)]	Response	[c)]BF05	Response	DF11	Response
Acaciacrassicarpa	4.00a	HS[c)]	3.48a	HS	3.96a	HS
Falcatariamoluccana	3.44ab	HS	3.04a	HS	2.80ab	S
Archidendronpauciflorum	1.96bc	MS	1.88b	MS	1.40cd	MS
Leucaenaleucocephala	1.52c	MS	1.56b	MS	1.68bc	MS
Parkiaspeciosa	1.80c	MS	1.04bc	MS	2.16bc	S
Acaciaauriculiformis	0.36d	MR	0.40c	MR	0.60d	MR

Valuesfollowedbythesameletterineachrowarenotsignificant.
[a]DI0–4,where0=nodisease/healthyseedling,1= yellowleaves,2=yellowleavesandslightlywilted,3=severewilt, and4=deadseedling.
[b)]*F.oxysporum*isolates.
[c)]Host responsegrouped as:R =resistant(DI=0); MR=moderatelyresistant/tolerance (DI = <1);MS=moderatelysusceptible(DI = 1.0–2.0);S=susceptible(DI=2.1–3.0);HS=highlysusceptible(DI=3.1–4.0)(Bertettietal.2018).

***Fusariumoxysporum*population**

Thetotalpopulationof*F.oxysporum*ontherootswasdeterminedbycalculatingtheCFUforeachcategoryof damage.ForDI4,*A.crassicarpa*and*F.moluccana*showedasignificantlyhigherpopulation(82.00–105.10×10^4CFUg^{-1}freshweight)thanotherplants.Thelowestpopulationwasrecordedin*P.speciosa*and*A.pauciflorum*(3.57–12.27× 10^4 CFUg^{-1}freshweight).Thissamepatternalsooccurredin DI2and3,whilenosamplewasrecordedin*A.auriculiformis* forDI2and3.InDI1,thehighestpopulationwasrecordedin*F.moluccana*and*L.leucocephala*,while*A.crassicarpa* and*A. auriculiformis*hadno sampleforDI1. Ininoculatedplants withDI0,the populationwas significantlyhigher in*L. leucocephala*and*A.auriculiformis*andnosamplewasnotedin*A.crassicarpa*and*F.moluccana*(Table2&Table3).

Theregressionanalysisresultsshowedthatallplantsexcept*P.speciosa*hadalinearrelationshippatternbetweenthe increaseindiseasescoreandpopulation.Thepathogenicpopulationon*A.crassicarpa*and*F.moluccana*grewrapidly along with theincreasein diseasescores,asindicated by themagnitudeofregression gradientcoefficient(m=20.3–21.3). However,moderateincreasewasobservedin*L.leucocephala*(m=11.2)(m=11.2)andveryslowin*A.pauciflorum*,*P. speciose*,and*A.auriculiformis*(m=2.2–4.8)(Figure8).

Table3showedthatisolateswerediffer entin*tef1-α*,butthepopulationandDIpatternsweresimilarforeachtestplant.Thecorrelation betweenthepopulationofpathogen(g^{-1}freshweight)andthe levelofDIweredescribedasfollows:i)highpathogen populations with

highDI(*A.crassicarpa*and*F.moluccana*),ii)moderatepopulationwithmoderateDI(*L.leucocephala*),iii)lowpopulationwith moderate DI (*A. pauciflorum*), and iv) low population with low DI (*P. speciosa* and *A. auriculiformis*).

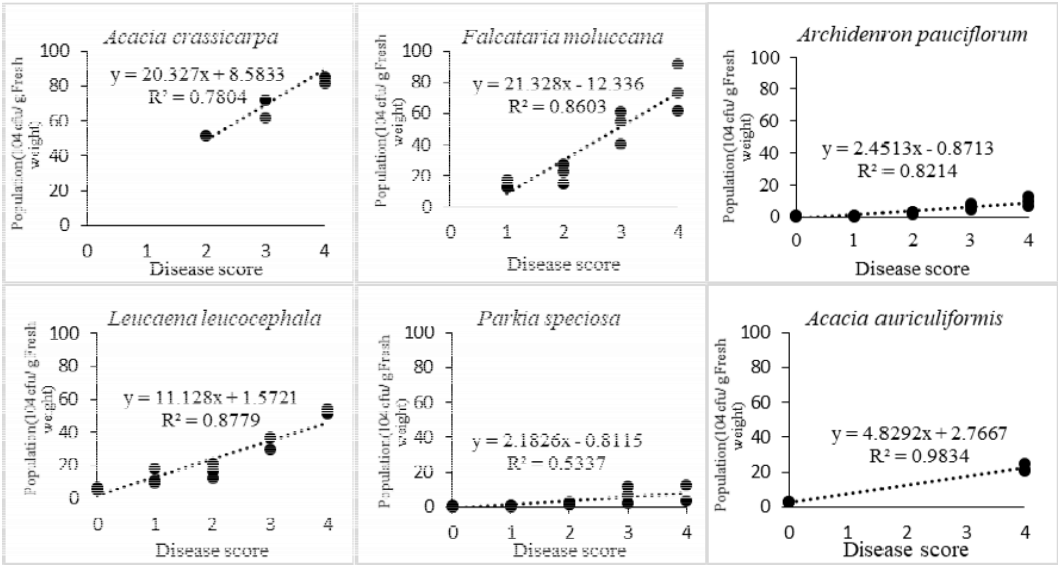

Figure8. Regressionanalysisofdiseasescorerateand*F.oxysporum*population

Table2. *Fusariumoxysporum* population on root in each disease index

Plant species	Population of *Fusarium oxysporum* ($\times 10^4$ CFU/g fresh weight)[a]					Average[c]
	0[b]	1	2	3	4	
AF01[d]						
Acacia crassicarpa	n.s	n.s	n.s	n.s	85.13a[e]	85.13
Falcataria moluccana	n.s	17.77a	22.77a	60.98a	91.87a	76.50
Archidendron pauciflorum	0.45b	1.10b	3.22b	8.15b	12.53cd	5.06
Leucaena leucocephala	6.17a	18.10a	20.93a	n.s	51.67b	22.13
Parkia speciosa	0.32b	0.45b	2.58b	7.27b	3.57d	2.16
Acacia auriculiformis	2.92a	n.s	n.s	n.s	24.53c	4.65
BF05						
Acacia crassicarpa	n.s	n.s	51.80a	72.08a	105.10a	92.61
Falcataria moluccana	n.s	13.22a	15.32b	40.33b	61.67b	43.85
Archidendron pauciflorum	0.47c	0.63b	1.73c	6.88c	9.90d	3.60
Leucaena leucocephala	4.67a	9.02a	12.32b	29.32b	n.s	11.16
Parkia speciosa	0.48c	0.57b	1.27c	2.33d	n.s	0.87
Acacia auriculiformis	2.55b	n.s	n.s	n.s	20.43c	3.98
DF11						
Acacia crassicarpa	n.s	n.s	n.s	61.92a	82.00a	81.20
Falcataria moluccana	n.s	12.50a	27.47a	54.93a	73.00a	47.93
Archidendron pauciflorum	0.35c	0.35b	3.37c	4.42c	6.92e	2.19
Leucaena leucocephala	5.58a	11.17a	16.53b	36.63b	54.27b	19.69
Parkia speciosa	0.25c	0.48b	1.58c	11.97d	12.27d	5.79
Acacia auriculiformis	2.83b	n.s	n.s	n.s	21.28c	5.05

n.s: No sample, cfu: colony forming unit

[a] *F. oxysporum* population calculated at the end of the experiment (21 days after inoculation).

[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] Average of *F. oxysporum* population (cfu/g fresh weight) = $(P_0A+P_1B+P_2C+P_3D+P_4E)/N$; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score 4; N = total number of plants.

[d] *F. oxysporum* isolates

[e] Values followed by the same letter in each row are not significant.

Table3. *Fusarium oxysporum* population average and diseases index of plant

Plant species	Population average ($\times 10^4$ CFU/g fresh weight) [a]			Disease index [b]		
	AF01[c]	BF05	DF11	AF01	BF05	DF11
Acacia crassicarpa	85.13	92.61	81.20	4.00	3.48	3.96
Falcataria moluccana	76.50	43.85	47.93	3.44	3.04	2.80
Archidendron pauciflorum	5.06	3.60	2.19	1.96	1.88	1.40
Leucaena leucocephala	22.13	11.16	19.69	1.52	1.56	1.68
Parkia speciosa	2.16	0.87	5.79	1.80	1.04	2.16
Acacia auriculiformis	4.65	3.98	5.05	0.36	0.40	0.60

[a] Average of *F. oxysporum* population (cfu/g fresh weight) = $(P_0A+P_1B+P_2C+P_3D+P_4E)/N$; where P0, P1, P2, P3, and P4 = population of pathogen in score 0, 1, 2, 3, and 4: A = number of plants on score 0; B = number of plants on score 1; C = number of plants on score 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.

[b] DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.

[c] *F. oxysporum* isolates.

Discussion

A recent study reported an extraordinary incidence of seedling wilt disease caused by fungal pathogen *F. oxysporum* attacking commercial nurseries of *A. mangium* in South Sumatra (Solehaet al. 2021). Therefore, the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Host range tests also provide information about plant species that have the potential to become alternative hosts or main hosts for the pathogen (Sampaio et al. 2021).

The results indicated that *F. oxysporum*, which causes vascular wilt in *A. mangium* nursery, can also infect fabaceae plants with various host responses. *A. crassicarpa* and *F. moluccana* were highly susceptible, while *A. pauciflorum*, *L. leucocephala*, and *P. speciosa* were moderately vulnerable, and *A. auriculiformis* was moderately resistant. Pathogen caused wilting symptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in *A. auriculiformis*, but it had the potential to damage plants. *F. oxysporum* able to infect plants even with a low DI, causing the death of cultivars. Moreover, when a plant is grown in contaminated soil, there is a high risk of damage to crops. A similar incident was reported by Pastrana et al. (2017) in which *F. oxysporum* from blackberry also caused sudden death in strawberries. Another study also revealed that *F. oxysporum* from cactus causes root and stem rot diseases in *Euphorbia* (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative hosts and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrence of new outbreaks because the pathogenic strains adapted to the soil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nursery activities that use contaminated soil repeatedly also triggered the pathogens proliferation and adaptation to other plants.

The pathogen population in *A. crassicarpa* and *F. moluccana* grew very rapidly with increasing disease scores, while in *L. leucocephala* grew moderately, and *A. pauciflorum*, *P. speciose*, and *A. auriculiformis* grew slowly. In this study, the population of *F. oxysporum* on highly susceptible plants (*A. crassicarpa* and *F. moluccana*) was significantly higher than other plants for each disease score. This pattern is common where the population of pathogen is also higher with disease scores (Scott et al. 2014). de Borba et al. (2017) reported that susceptible lettuce cultivars showed high *Fusarium* population level and vulnerable black bean genotype showed a population level of 15.4×10^5 CFU $^{g-1}$. The second pattern was observed on *L. leucocephala*, where the population of pathogen was also moderate with a moderate disease sesscore. The similar result was also occurred in garlic with a disease severity of 44% due to *Fusarium* spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

A special pattern occurred on *A. pauciflorum* in which *F. oxysporum* caused a moderate infection, but the pathogen population was low. This might be due to the plant defence mechanism. Scott et al. (2014) reported that resistant pepper plants also support pathogen development in roots, even without external symptoms. Similar phenomena was reported by Muslim et al. (2003a) who noted that some tomato plants are infected moderately (score 1–2) by *F. oxysporum* f.sp. *lycopersici*, but the population was lower than other plants in same score.

The infection and total population on *Parkia speciosa* and *A. auriculiformis* was lower. This indicated that plants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with *F. oxysporum* f.sp. *fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonisation and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited to the epidermis, then the pathogens do not reach the vascular tissue. Van Den Berg et al. (2007) reported that banana clones tolerant to *F. oxysporum* f.sp. *cubense* correspond with this, with a significant increase in the

inductionofcellwall-associatedphenoliccompounds.Jiménez-Fernándezetal.(2013)alsoreportedthat*Fusarium oxysporum*f.sp.*ciceris*race0remainedintheintercellularspaceofrootcortexandfailedtoreachxyleminresistant chickpeacultivars.

Inthisstudy,*A.crassicarpa*and*F.moluccana*wereproventobeanalternativehostof*F.oxysporum*.Whereas*L. leucocephala*,*A.pauciflorum*,*P.speciosa*,and*A.auriculiformis*hadpotentialasalternativehosts.Manyplantsof fabaceaefamilywasattackedbyformaespecialis*F.oxysporum*,suchas*Vignaangularis*(*F.oxysporum*f.sp.*adzukicola*), *Cicerarietinum*,*Cicer*spp.(*F.oxysporum*f.sp.*ciceris*),*Acacia*spp.(*F.oxysporum*f.sp.*koae*),*Lensculinaris*,*L. esculenta*(*F.oxysporum*f.sp.*lentis*),*Medicagosativa*(*F.oxysporum*f.sp.*medicaginis*),*Phaseolusvulgaris*, *P. coccineus*(*F.oxysporum*f.sp.*phaseoli*),*Pisumsativum*,*Cicerarietinum*(*F.oxysporum*f.sp.*pisi*)(Edel-Hermannand Lecomte2019).However,inthisstudy,*F.oxysporum*isolatedfrom*A.mangium*hasawidehostrangefromfabaceae family;therefore,itisnotclassifiedasformaespecialis.

In conclusion,*F.oxysporum*isolated from*A. mangium*causesinfection in severaltypesof forestand industrial plants. Sinceithasawidehostrange,itisnotclassifiedaspartoftheformaespecialisgroup.

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Pleasefeelfreetocontactmeifyouneedanyadditionalinformationorclarification. Thank you very much for your excellent cooperation

Sincerely,
AhmadMuslim
Senior lecturer
FacultyofAgriculture,SriwijayaUniversity
Jl.Palembang-PrabumulihKm.32,Indralaya,Palembang,Indonesia E-

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Jl.Palembang-PrabumulihKm.32,Indralaya,Palembang,Indonesia

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Hostrangestudiesof*Fusariumoxysporum*,causalagentofseedlingwilt disease of *Acacia mangium*

SOLEHASOLEHA[1],AHMADMUSLIM[2,♥],SUWANDISUWANDI[2],SABARUDDINKADIR[3],RAHMAT PRATAMA[1]

[1]ProgramofAgricultureSciences,FacultyofAgriculture,UniversitasSriwijaya.Jl.IndralayaIndah,Indralaya,OganIlirRegency,SumatraSelatan,Indonesia
[2]DepartmentofPlantProtection,FacultyofAgriculture,UniversitasSriwijaya.Jl.IndralayaIndah,Indralaya,OganIlirRegency,SumatraSelatan,Indonesia, Tel./fax. +62-896-3874-9695 ♥email: a_muslim@unsri.ac.id
[3]DepartmentofSoilSciences,FacultyofAgriculture,UniversitasSriwijaya,Jl.IndralayaIndah,Indralaya,OganIlirRegency,SumatraSelatan,Indonesia

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Abstract.*SolehaS,MuslimA,SuwandiS,KadirS,PratamaR.2021.Hostrangestudiesof*Fusariumoxysporum,*causalagentof seedlingwiltdiseaseof* Acaciamangium.*Biodiversitas 23:xxxx.Fusariumoxysporum*isaseriouspathogen thatcausesseverewilt diseaseincommercialnurseriesof*Acaciamangium*inSouthSumatra.Thisstudyaimedtoinvestigatethehostrangeof*F.oxysporum*as a nursery wilt pathogen in*A. mangium* and several forest and industrial plants. Three isolatesof*F. oxysporum* with different translation elongationfactor(*tef*1-□)sequencesweretestedforpathogenicityondifferentfabaceaefamilyplantsandthegrowthofpopulationwas alsoobserved.Theresultsshowedthatallthethreeisolateswereabletoinfectallthetestedplantswithdifferentreactionsofwilt disease.The *Acaciacrassicarpa*and *Falcatariamoluccana*werehighlysusceptible;*Archidendronpauciflorum*,*Leucaena leucocephala*,and *Parkiaspeciosa* weremoderatelyvulnerableand *Acaciaauriculiformis*wasmoderatelyresistant.Thepathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, whilein*L. leucocephala* it was moderate,andslowin *A.pauciflorum*, *P.speciosa*and *A.auriculiformis* plants.Inconclusion, *F.oxysporum* pathogen,whichwas isolated from *A. mangium*, has a wide range of hosts in the fabaceae family.

Keyword:*Acaciamangium*,fabaceae,*Fusariumoxysporum*,hostrange,seedlingwilt

INTRODUCTION

Acacia mangium (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea,andAustralia,andwhich,hasalsobeenfoundfora few decadesin the humid tropical lowlandsof Asia, South America, and Africa (Koutika and Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to variousenvironmentalconditions(Asif et al. 2017), itiswidely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

*Fusariumoxysporum*isanimportantpathogenicfungus that causes wilt disease in different plants all over the world.Soleha et al. (2021) reported that it was identifiedas the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra.The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Rana et al. 2017; Muslim et al. 2019). Furthermore, it attacks almost every type of plant, from cultivatedtoforestandwild(e.g.weeds)(Joshi2018).This fungusisalsoabletoattackvariousplanthabitssuchas trees(Zhangetal.2013),herbaceousplants(Jacobsand Heerden 2012), and vines (Rooney-Latham and Blomquist 2011).Severaltypesofforestplantsthathavereportedly been attacked by*F. oxysporum* are*Pinus massoniana*(Luo andYu2020), *Tectonagrandis* (Borgesetal.2018), *Pseudotsugamenziesii* (Stewartetal.2011), *Acacia mangium* (Widyastuti et al. 2013) and others.

Since*F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). According to Leslie and Summerell (2006) more than 100 formae species and races have been identified and are widespread in the world.

Besides *A.mangium*,whichisthemainplantof industrialforestryinIndonesia,otherplants,suchas*Acacia crassicarpa*, *Acaciaauriculiformis*, *Parkiaspeciosa*, *Archidendronpauciflorum*, *Falcataria moluccana*,and *Leucaenaleucocephala*arealsoimportantandhavehigh economic value. Considering that they belong to the same family(Fabaceae),theycanbecomethemainoralternative hostsfor *F.oxysporum*, causativeagentofwiltdisease. Thisstudyaimedtoinvestigatethehostrangeof *F.oxysporum* as a nursery wilt pathogen in*A. mangium* and several industrial and local forest plants in Indonesia.

MATERIALSANDMETHOD

Fungalisolates

Threepathogenicisolatesof *F.oxysporum*(AF01, BF05, and DF11) were selected, which were differentiated accordingtotheir *tef*1-αsequence(Figure1).Isolates wereculturedonPDBliquidmedium(potatodextrose broth) and incubated at 26-28°C on a shaker (150 rpm) for about five days. Then the mycelia suspension was filtered usingtwolayersofsterilegauzetoseparatetheconidiaand hyphae.Theconidialconcentrationwasdeterminedusinga hemocytometer and then adjusted to a concentration of 10^6 ml^{-1} for pathogenicity test.

Plantmaterial

The plants used were members of the fabaceae family, namely*A. crassicarpa, A. auriculiformis,F. moluccana,A. pauciflorum,P. speciosa*, and*L. leucocephala*, which were one month old. The seedlings were obtained from theForest Crops Research Institute, South Sumatra.Seedlings were transferred in a mixed medium with cocopeat (1:1) usingaplasticpotof10cmdiameterand10cmheight,and then placed in a shade house.

Pathogenicitytest

A pathogenicity test was carried out using root dip method, in which the roots were washed under running water and then immersed in 250 ml of conidia suspension (10^6 conidia ml^{-1}) for 15 minutes. The control plants were immersed in sterile distilled water, and the seedlings were transplanted into plastic pots and placed under a house shade. Each isolate was inoculated on 25 plants with five replicates (five plants per-replicate). Then, disease severity wascalculatedusingthemethodofMuslimetal.(2003a) and the disease index(DI) was classified into following grades, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling (Bertetti et al. 2018). Furthermore, plant responses were grouped as, R =resistant(DI=0),MR=moderatelyresistant/tolerance(DI = <1), MS = moderately susceptible (DI = 1.0–2.0), S = susceptible(DI = 2.1–3.0) andHS= highly susceptible(DI =3.1–4.0).Thedevelopmentofdiseasewasobserved1–21 days after inoculation.

*Fusariumoxysporum*population

Thepopulationof *F.oxysporum* intherootswas calculated atthe end ofthe experimentusingthe methodof (Muslimetal.2003b;Lietal.2008;Horinouchietal. 2011)withmodificationstothesurfacesterilizationof samples.Thentheplantsweregroupedaccordingto severity(diseasescore)andwashedseparatelyunder running water to remove soil residues. After that, all plants ineachscoreweresurfacesterilisedusing1%sodium hypochloritefor15minutes,thenrinsedthreetimeswith distilledwater.Thesamplesandwater(1:100w/v)were homogenised usingblender at8000 rpm for 10 minutes. Thentheywerefilteredusingtwolayersofsterilegauze anddiluted 10 to1000 times. The suspension was spread onPeptonePCNBagarMedia(PPA/NashSnyderMedium) (Leslie and Summerell 2006) in triplicate (five Petri dishes perreplication)andincubatedindarkforsevendaysat roomtemperature.Thenumberofcolony-formingunits (CFU)of*F.oxysporum*wascalculatedonthebasisoffresh weightpergramofsampleandgroupedaccordingtothe level of diseases severity.

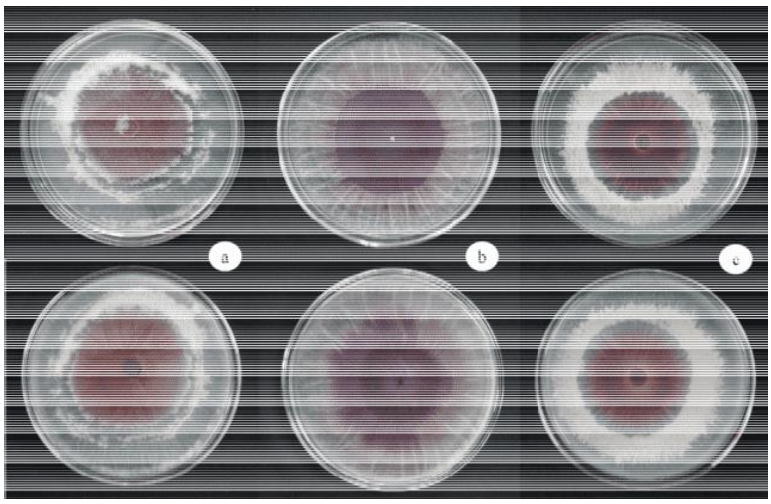

Figure1.*F.oxysporum*isolatesonPDAmedium.(a)AF01,(b)BF05,and(c)DF11.Firstline:frontview;secondline:reverseview.

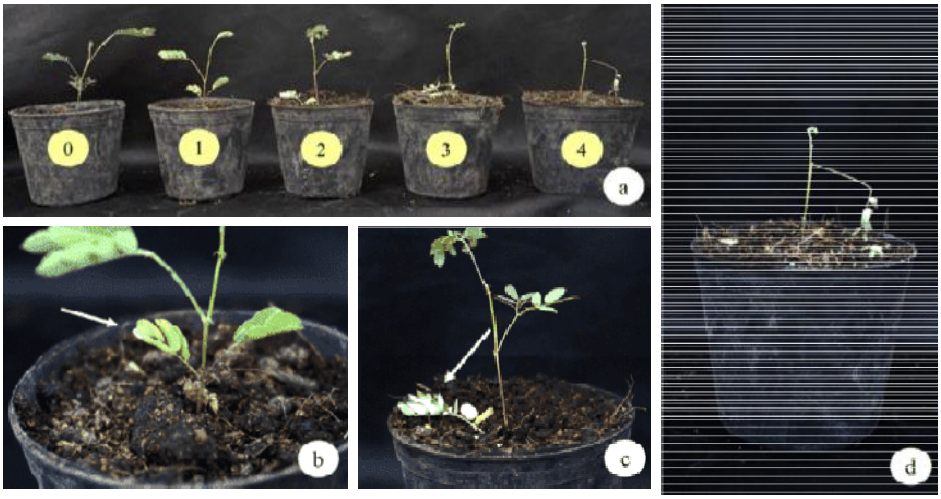

Figure 2. Disease index of*Acaciacrassicarpa*,(a) from left:healthy plantto 100% wilted leaves (score 0−4); (b) initial symptoms:yellowing from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

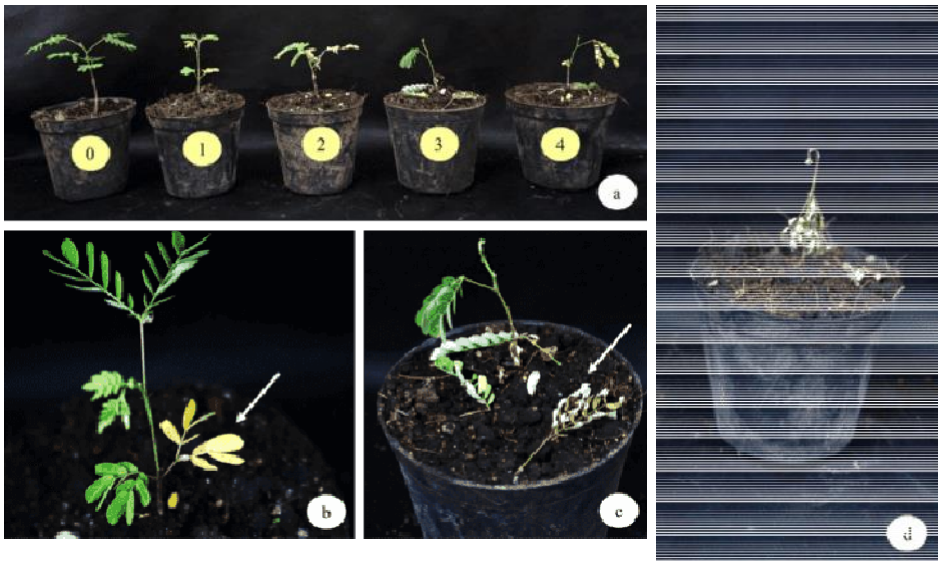

Figure 3. Disease index on*Falcataria moluccana*,(a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved, dry, and falling leaves; (d) dead plant

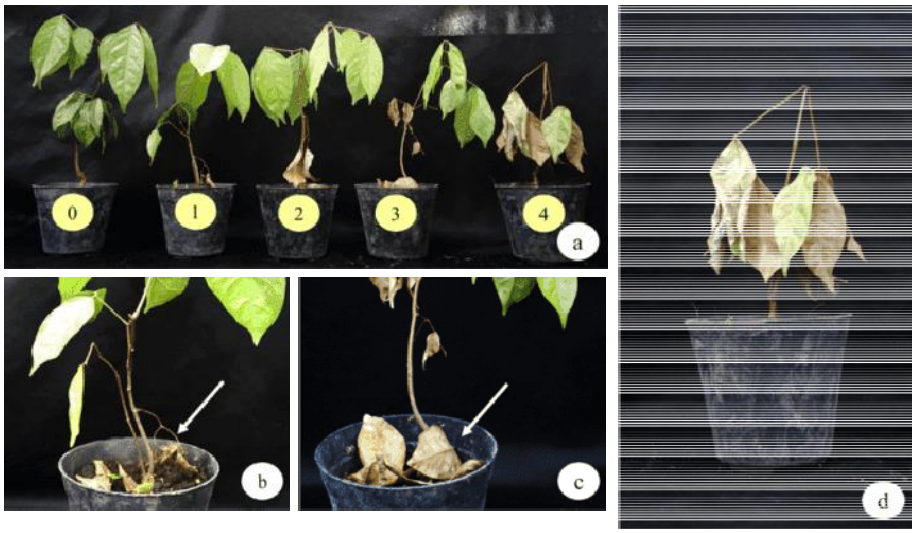

Figure4. Diseaseindexon*Archidendronpauciflorum*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

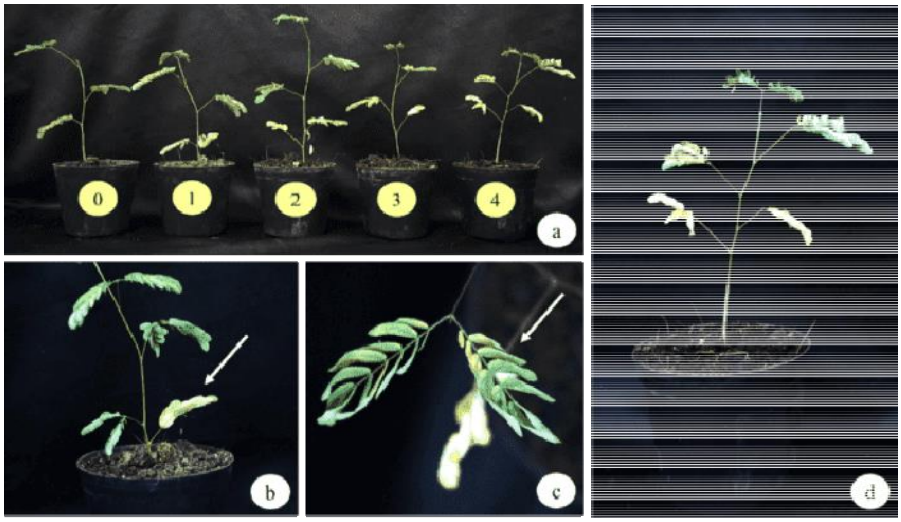

Figure5. Diseaseindexon*Leucaenaleucocephala*,(a)fromleft:healthyplant to100%wiltedleaves(score0−4);(b)initialsymptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward

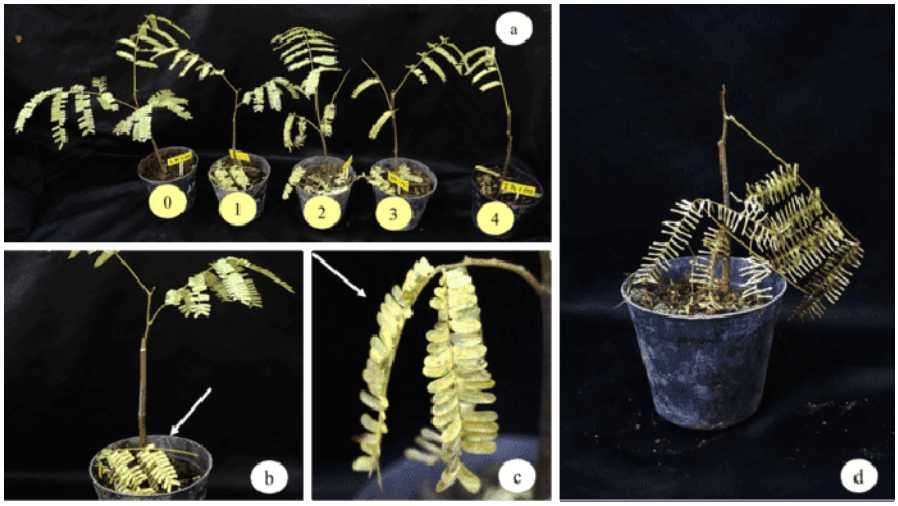

Figure6. Diseaseindexon*Parkiaspeciosa*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initialsymptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant

Figure7. Diseaseindexon*Acaciaauriculiformis*,fromleft:healthyplanttowiltedanddeadplant(score0–4)

RESULTSANDDISCUSSION

Pathogenicitytest

The results showed that all the six forest plants tested had similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of*F. oxysporum*infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progress to younger shoots, severe wilting, drying, falling of leaves, and eventually plant die. Another symptom that appeared was sudden wilting and death of plant without changing theleaf colour, while control plants did not show any symptoms (Figures 2-7).

Diseaseseveritywassignificantlyhigherthancontrols. *A.crassicarpa*and *F.moluccana*weremostseverely affectedwithanaveragescoreof4.00and3.44,respectively.Ontheotherhand, *A.pauciflorum*, *L. leucocephala*,and *P.speciosa*wereshowed moderate diseaseseverity i.e.1.96,1.68,and1.80,respectively, whereas*A.auriculiformis*hadthelowest(0.36)disease severity (Table 1).Based on the disease score, host plants were classified into three groups: i) highly susceptible (*A. crassicarpa* and*F. moluccana*), ii) moderately susceptible (*A. pauciflorum*P. *speciosa*, and*L. leucocephala*), and iii) moderateresistance/tolerance(*A.auriculiformis*).Result exhibited that there was no significant difference between thediseaseseverityinthesamehostthathadbeen inoculated with different isolates (Table 1).

Table1. Diseaseseverityandhostresponsesto*Fusariumoxysporum*isolatedfrom*Acaciamangium*

Plantspecies	Isolates[a)]					
	AF01[b)]	Response[c)]	BF05	Response	DF11	Response
Acaciacrassicarp	4.00a	HS[c)]	3.48	HS	3.96a	HS
Falcatariamoluccan	3.44ab	HS	3.04	HS	2.80ab	S
Archidendronpaucifloru	1.96b	MS	1.88b	MS	1.40cd	MS
Leucaenaleucocephal	1.52	MS	1.56b	MS	1.68bc	MS
Parkiaspecios	1.80	MS	1.04b	MS	2.16bc	S
Acaciaauriculiformi	0.36d	MR	0.40	MR	0.60d	MR

Valuesfollowedbythesameletterineachrowarenotsignificant.

[a]DI 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling.

[b)]*F.oxysporum*isolates.

[c)] Host response grouped as: R = resistant (DI = 0); MR = moderately resistant/tolerance (DI = <1); MS = moderately susceptible (DI = 1.0–2.0); S = susceptible (DI = 2.1–3.0); HS = highly susceptible (DI = 3.1–4.0) (Bertetti et al. 2018).

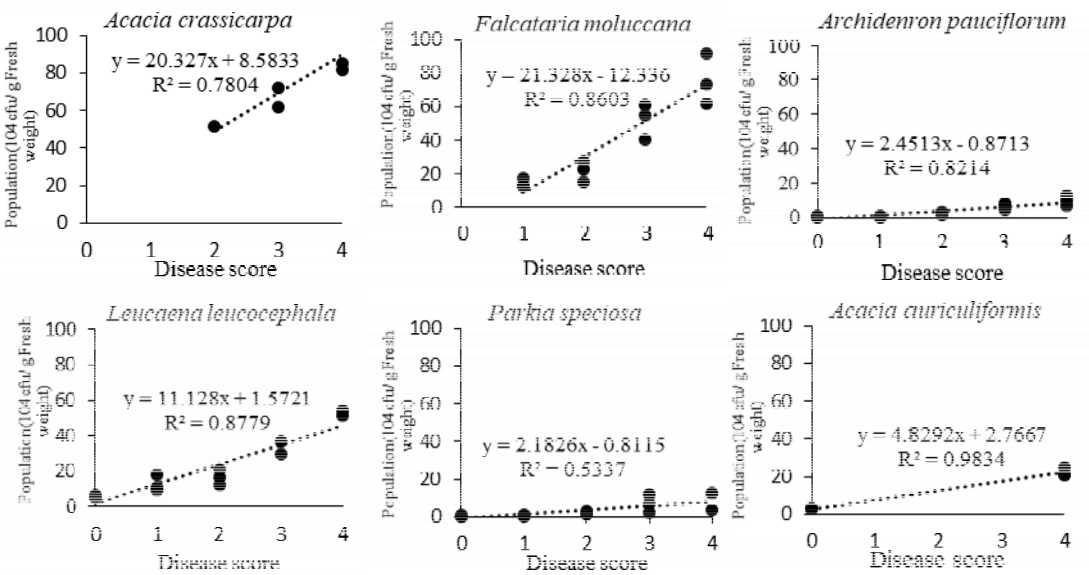

Figure8. Regressionanalysisofdiseasescorerateand*F.oxysporum*population

Table2.*Fusariumoxysporum*populationonrootineachdiseaseindex

Plantspecies	Populationof*Fusariumoxysporum*(×10^4CFU/gfreshweight)[a]					Average[c]
	0[b]	1	2	3	4	
AF01[d]						
Acaciacrassicarpa	n s	n.s	n.s	n.s	8513 a[e]	8513
Falcatariamoluccan	n.	1777	2277 a	6098 a	9187 a	7650
Archidendronpaucifloru	045b	110b	322b	815b	1253 cd	5 06
Leucaenaleucocephal	617a	1810 a	2093 a	n.s	5167b	2213
Parkiaspecios	032b	045b	258b	727b	357d	2 16
Acaciaauriculiformi	292a	n.	n.	n.	2453 c	4 65
BF05						
Acaciacrassicarp	n.	n.	5180 a	7208 a	10510	9261
Falcatariamoluccan	n.	1322	1532b	4033b	6167b	4385
Archidendronpaucifloru	047c	063b	173c	688c	990d	3 60
Leucaenaleucocephal	467a	902a	1232b	2932b	n.	1116
Parkiaspecios	0 48	057b	1 27	233d	n.	0 87
Acaciaauriculiformis	255b	n.s	n s	n.s	2043 c	3 98
DF11						
Acaciacrassicarp	n.	n.	n.	6192 a	8200 a	8120
Falcatariamoluccan	n.s	1250 a	2747 a	5493 a	7300 a	4793
Archidendronpaucifloru	0 35	035b	3 37	4 42	6 92	2 19
Leucaenaleucocephal	558a	1117	1653b	3663b	5427b	1969
Parkiaspecios	025c	048b	158c	1197d	1227d	5 79
Acaciaauriculiformi	283b	n.	n.	n.	2128 c	5 05

n.s:Nosample,cfu:colonyformingunit

[a]*F.oxysporum*populationcalculatedattheendoftheexperiment(21daysafterinoculation).

[b]DI0–4;0=nodisease/healthyseedling;1=yellowleaves;2=yellowleavesandslightlywilted;3=severewilt;and4=deadseedling.

[c]Averageof*F.oxysporum*population(cfu/gfreshweight)=($P_0A+P_1B+P_2C+P_3D+P_4E$)/N;wherePO,P1,P2,P3,andP4=populationof pathogeninscore0, 1,2,3,and4:A=number of plantson score0; B =number of plantson score1; C =number ofplantson score2; D =number ofplantson score3; E = number ofplantson score4; N = totalnumber of plants.

[d]*F.oxysporum*isolates

[e]Valuesfollowedbythesameletterineachrowarenotsignificant.

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Table3.*Fusariumoxysporum*populationaverageanddiseasesindexofplant

Plantspecies	Populationaverage(×10^4CFU/gfresh weight)[a)]			Diseaseindex[b)]		
	AF01[c)]	BF05	DF11	AF01	BF05	DF11
Acaciacrassicarp	8513	9261	8120	4.00	3.48	3.96
Falcatariamoluccan	7650	4385	4793	3.44	3.04	2.80
Archidendronpaucifloru	5 06	3.60	2.19	1.96	1.88	1.40
Leucaenaleucocephal	2213	1116	1969	1.52	1.56	1.68
Parkiaspecios	2 16	0.87	5.79	1.80	1.04	2.16
Acaciaauriculiformi	4 65	3.98	5.05	0.36	0.40	0.60

[a)]Average of*F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population ofpathogenin score0,1,2,3,and4: A=numberofplantson score 0;B= numberofplantsonscore 1;C= numberofplantsonscore 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.
[b)]DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.
[c)]*F.oxysporum*isolates.

Discussion

Arecentstudyreportedanextraordinaryincidenceof seedlingwiltdiseasecausedbyfungalpathogen *F. oxysporum* attacking commercial nurseries of*A. mangium* inSouthSumatra(Solehaetal.2021).Therefore,the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Hostrangetestsalsoprovideinformationabout plant species that have the potential to become alternative hosts or main hosts for the pathogen (Sampaio et al. 2021).

Theresults indicated that*F. oxysporum*,which causes vascularwiltin*A.mangium*nursery,canalsoinfect fabaceae plantswith varioushostresponses.*A. crassicarpa* and *F.moluccana*werehighlysusceptible,while *A. pauciflorum*, *L.leucocephala*,and *P.speciosa*were moderately vulnerable, and *A. auriculiformis* was moderatelyresistant.Pathogencausedwiltingsymptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in*A. auriculiformis*, but it had the potential to damageplants. *F.oxysporum*abletoinfectplantseven withalowDI,causingthedeathofcultivars.Moreover, when a plant is grown in contaminated soil, there is a high risk ofdamageto crops. Asimilar incidentwasreportedby Pastranaetal.(2017)inwhich *F.oxysporum*from blackberryalsocausedsuddendeathinstrawberries. Another study also revealed that*F. oxysporum* from cactus causes root and stem rot diseasesin*Euphorbia* (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative hosts and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrenceofnewoutbreaksbecausethepathogenicstrains adapted to the soil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nurseryactivitiesthatusecontaminatedsoilrepeatedlyalso triggered the pathogens proliferation and adaptation toother plants.

Thepathogenpopulationin *A.crassicarpa*and *F. moluccana*grewveryrapidlywithincreasingdisease scores,whilein*L.leucocephala*grewmoderately, and *A. pauciflorum*, *P.speciose*,and *A.auriculiformis*grew slowly.Inthisstudy,thepopulationof*F.oxysporum*on highly susceptible plants (*A. crassicarpa* and *F. moluccana*)wassignificantlyhigherthanotherplantsfor eachdiseasescore.Thispatterniscommonwherethe populationofpathogenisalsohigherwithdiseasescores (Scottetal.2014).deBorbaetal.(2017)reportedthat susceptiblelettucecultivarsshowedhigh *Fusarium* populationlevelandvulnerableblackbeangenotype showedapopulationlevelof15.4×10^5CFU^{g-1}.The second patternwasobservedon*L. leucocephala*, where the population of pathogen was also moderate with a moderate diseasesscore.Thesimilarresultwasalsooccurredin garlic with a disease severity of 44% due to*Fusarium* spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

Aspecialpatternoccurredon*A.pauciflorum*inwhich *F.oxysporum*causedamoderateinfection,butthe pathogenpopulationwaslow.Thismightbeduetothe plant defence mechanism. Scott et al. (2014)reported that resistant pepper plants also support pathogen development inroots,evenwithoutexternalsymptoms.Similarphenomenaw asreportedbyMuslimetal.(2003a)who notedthatsometomatoplantsareinfectedmoderately (score1–2)by *F.oxysporum*f.sp. *lycopersici*,butthe population was lower than other plants in same score.

The infection and total population on*Parkia speciosa* and*A. auriculiformis* was lower. Thisindicated thatplants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with*F. oxysporum* f. sp.*fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonisation and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited tothe epidermis, then the pathogens do notreach the vascular tissue. Van Den Berg et al. (2007) reported that banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond withthis,with asignificantincrease intheinductionof cell

wall-associated phenolic compounds. Jiménez-Fernándezet al. (2013) also reported that*Fusarium oxysporum* f. sp. *ciceris* race 0 remained in the intercellular space of root cortex and failed to reach xylem in resistant chickpea cultivars.

Inthisstudy, *A.crassicarpa*and *F.moluccana* were proven to be an alternative host of*F.oxysporum.* Whereas *L.leucocephala*, *A.pauciflorum*, *P.speciosa*,and *A. auriculiformis*hadpotentialasalternativehosts.Many plantsoffabaceae familywasattackedbyformaespecialis *F.oxysporum*,suchas*Vignaangularis*(*F.oxysporum*f.sp. *adzukicola*),*Cicerarietinum*,*Cicer*spp.(*F.oxysporum*f. sp. *ciceris*), *Acacia*spp.(*F.oxysporum* f.sp. *koae*), *Lens culinaris*,*L.esculenta*(*F.oxysporum* f.sp. *lentis*), *Medicagosativa*(*F.oxysporum* f.sp. *medicaginis*), *Phaseolusvulgaris*, *P.coccineus*(*F.oxysporum* f.sp. *phaseoli*),*Pisumsativum*,*Cicerarietinum*(*F.oxysporum*f. sp.*pisi*) (Edel-Hermann and Lecomte 2019). However, in this study,*F. oxysporum* isolatedfrom *A.mangium* has a wide host range fromfabaceae family; therefore, it is not classified as formae specialis.

In conclusion,*F. oxysporum*isolated from*A. mangium* causes infection in several types of forest and industrial plants.Since it has a wide host range, it is not classified as part of the formae specialis group.

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Hostrangestudiesof*Fusariumoxysporum*,causalagentofseedlingwilt disease of *Acacia mangium*

SOLEHASOLEHA[1],AHMADMUSLIM[2,♥],SUWANDISUWANDI[2],SABARUDDINKADIR[3],RAHMAT PRATAMA[1]

[1]ProgramofAgricultureSciences,FacultyofAgriculture,UniversitasSriwijaya.Jl.IndralayaIndah,Indralaya,OganIlirRegency,SumatraSelatan,Indonesia
[2]DepartmentofPlantProtection,FacultyofAgriculture,UniversitasSriwijaya.Jl.IndralayaIndah,Indralaya,OganIlirRegency,SumatraSelatan,Indonesia, Tel./fax. +62-896-3874-9695 ♥email: a_muslim@unsri.ac.id
[3]DepartmentofSoilSciences,FacultyofAgriculture,UniversitasSriwijaya,Jl.IndralayaIndah,Indralaya,OganIlirRegency,SumatraSelatan,Indonesia

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Abstract.*SolehaS,MuslimA,SuwandiS,KadirS,PratamaR.2021.Hostrangestudiesof*Fusariumoxysporum,*causalagentof seedlingwiltdiseaseof* Acaciamangium.*Biodiversitas 23:xxxx.Fusariumoxysporum*isaseriouspathogen thatcausesseverewilt diseaseincommercialnurseriesof*Acaciamangium*inSouthSumatra.Thisstudyaimedtoinvestigatethehostrangeof*F.oxysporum*as a nursery wilt pathogen in*A. mangium* and several forest and industrial plants. Three isolatesof*F. oxysporum* with different translation elongationfactor(*tef*1-□)sequencesweretestedforpathogenicityondifferentfabaceaefamilyplantsandthegrowthofpopulationwas alsoobserved.Theresultsshowedthatallthethreeisolateswereabletoinfectallthetestedplantswithdifferentreactionsofwilt disease.The *Acaciacrassicarpa*and *Falcatariamoluccana*werehighlysusceptible;*Archidendronpauciflorum*,*Leucaena leucocephala*,and *Parkiaspeciosa* weremoderatelyvulnerableand *Acaciaauriculiformis*wasmoderatelyresistant.Thepathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, whilein*L. leucocephala* it was moderate,andslowin *A.pauciflorum*, *P.speciosa*and *A.auriculiformis* plants.Inconclusion, *F.oxysporum* pathogen,whichwas isolated from *A. mangium*, has a wide range of hosts in the fabaceae family.

Keyword:*Acaciamangium*,fabaceae,*Fusariumoxysporum*,hostrange,seedlingwilt

INTRODUCTION

Acacia mangium (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea,andAustralia,andwhich,hasalsobeenfoundfora few decadesin the humid tropical lowlandsof Asia, South America, and Africa (Koutika and Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and high adaptability to variousenvironmentalconditions(Asif et al. 2017), itiswidely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

*Fusariumoxysporum*isanimportantpathogenicfungus that causes wilt disease in different plants all over the world.Soleha et al. (2021) reported that it was identifiedas the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra.The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Rana et al. 2017; Muslim et al. 2019). Furthermore, it attacks almost every type of plant, from cultivatedtoforestandwild(e.g.weeds)(Joshi2018).This fungusisalsoabletoattackvariousplanthabitssuchas trees(Zhangetal.2013),herbaceousplants(Jacobsand Heerden 2012), and vines (Rooney-Latham and Blomquist 2011).Severaltypesofforestplantsthathavereportedly been attacked by*F. oxysporum* are*Pinus massoniana*(Luo andYu2020), *Tectonagrandis* (Borgesetal.2018), *Pseudotsugamenziesii* (Stewartetal.2011), *Acacia mangium* (Widyastuti et al. 2013) and others.

Since*F. oxysporum* has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Taylor et al. 2019). According to Leslie and Summerell (2006) more than 100 formae species and races have been identified and are widespread in the world.

Besides *A.mangium*,whichisthemainplantof industrialforestryinIndonesia,otherplants,suchas*Acacia crassicarpa*, *Acaciaauriculiformis*, *Parkiaspeciosa*, *Archidendronpauciflorum*, *Falcataria moluccana*,and *Leucaenaleucocephala*arealsoimportantandhavehigh economic value. Considering that they belong to the same family(Fabaceae),theycanbecomethemainoralternative hostsfor *F.oxysporum*, causativeagentofwiltdisease. Thisstudyaimedtoinvestigatethehostrangeof *F.oxysporum* as a nursery wilt pathogen in*A. mangium* and several industrial and local forest plants in Indonesia.

MATERIALSANDMETHOD

Fungalisolates

Threepathogenicisolatesof *F.oxysporum*(AF01, BF05, and DF11) were selected, which were differentiated accordingtotheir *tef*1-αsequence(Figure1).Isolates wereculturedonPDBliquidmedium(potatodextrose broth) and incubated at 26-28°C on a shaker (150 rpm) for about five days. Then the mycelia suspension was filtered usingtwolayersofsterilegauzetoseparatetheconidiaand hyphae.Theconidialconcentrationwasdeterminedusinga hemocytometer and then adjusted to a concentration of 10^6 ml^{-1} for pathogenicity test.

Plantmaterial

The plants used were members of the fabaceae family, namely*A. crassicarpa, A. auriculiformis,F. moluccana,A. pauciflorum,P. speciosa*, and*L. leucocephala*, which were one month old. The seedlings were obtained from theForest Crops Research Institute, South Sumatra.Seedlings were transferred in a mixed medium with cocopeat (1:1) usingaplasticpotof10cmdiameterand10cmheight,and then placed in a shade house.

Pathogenicitytest

A pathogenicity test was carried out using root dip method, in which the roots were washed under running water and then immersed in 250 ml of conidia suspension (10^6 conidia ml^{-1}) for 15 minutes. The control plants were immersed in sterile distilled water, and the seedlings were transplanted into plastic pots and placed under a house shade. Each isolate was inoculated on 25 plants with five replicates (five plants per-replicate). Then, disease severity wascalculatedusingthemethodofMuslimetal.(2003a) and the disease index(DI) was classified into following grades, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling (Bertetti et al. 2018). Furthermore, plant responses were grouped as, R =resistant(DI=0),MR=moderatelyresistant/tolerance(DI = <1), MS = moderately susceptible (DI = 1.0–2.0), S = susceptible(DI = 2.1–3.0) andHS= highly susceptible(DI =3.1–4.0).Thedevelopmentofdiseasewasobserved1–21 days after inoculation.

*Fusariumoxysporum*population

Thepopulationof *F.oxysporum* intherootswas calculated atthe end ofthe experimentusingthe methodof (Muslimetal.2003b;Lietal.2008;Horinouchietal. 2011)withmodificationstothesurfacesterilizationof samples.Thentheplantsweregroupedaccordingto severity(diseasescore)andwashedseparatelyunder running water to remove soil residues. After that, all plants ineachscoreweresurfacesterilisedusing1%sodium hypochloritefor15minutes,thenrinsedthreetimeswith distilledwater.Thesamplesandwater(1:100w/v)were homogenised usingblender at8000 rpm for 10 minutes. Thentheywerefilteredusingtwolayersofsterilegauze anddiluted 10 to1000 times. The suspension was spread onPeptonePCNBagarMedia(PPA/NashSnyderMedium) (Leslie and Summerell 2006) in triplicate (five Petri dishes perreplication)andincubatedindarkforsevendaysat roomtemperature.Thenumberofcolony-formingunits (CFU)of*F.oxysporum*wascalculatedonthebasisoffresh weightpergramofsampleandgroupedaccordingtothe level of diseases severity.

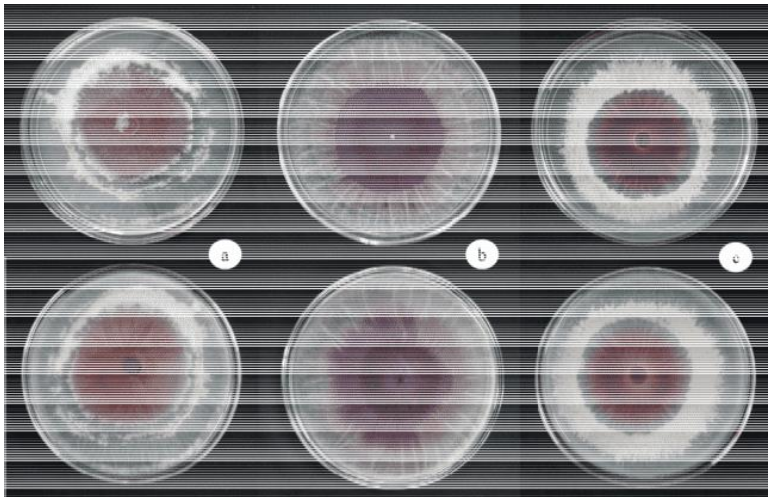

Figure1.*F.oxysporum*isolatesonPDAmedium.(a)AF01,(b)BF05,and(c)DF11.Firstline:frontview;secondline:reverseview.

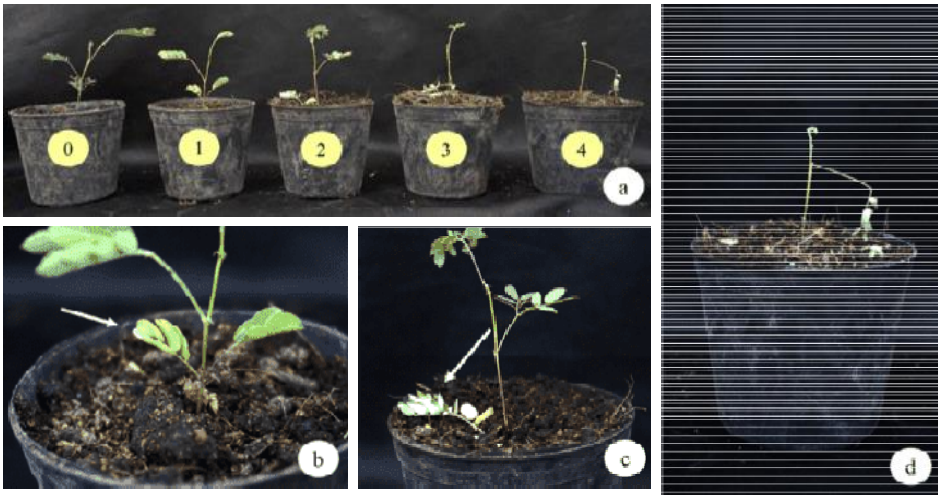

Figure 2. Disease index of*Acaciacrassicarpa*,(a) from left:healthy plantto 100% wilted leaves (score 0−4); (b) initial symptoms:yellowing from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

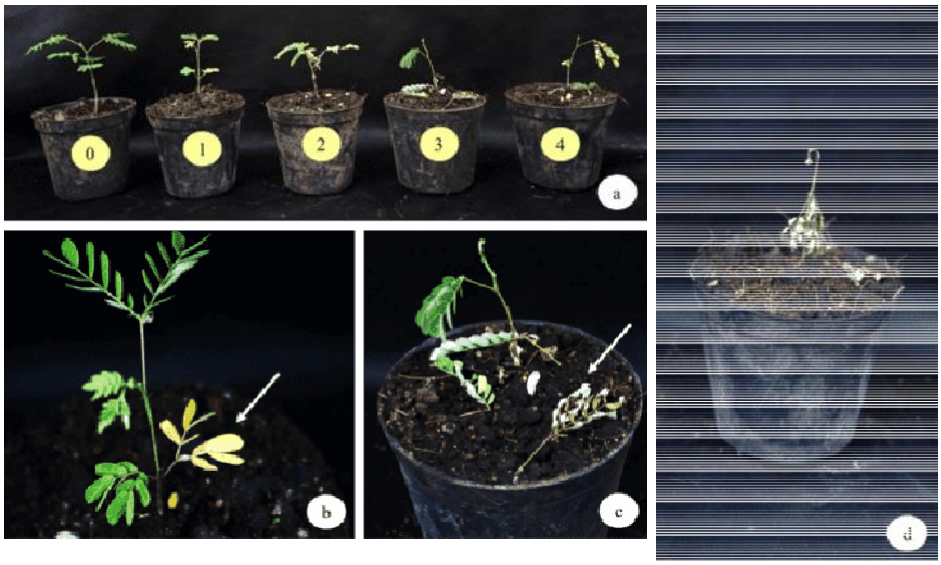

Figure 3. Disease index on*Falcataria moluccana*,(a) from left: healthy plant to 100% wilted leaves (score 0−4); (b) initial symptoms: yellowing from oldest leaves; (c) advanced symptoms: curved, dry, and falling leaves; (d) dead plant

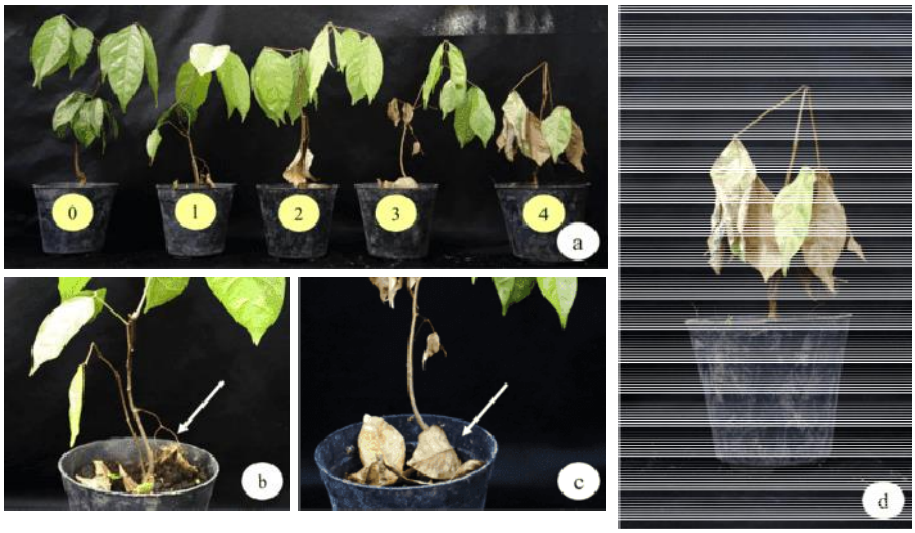

Figure4. Diseaseindexon*Archidendronpauciflorum*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initial symptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: falling leaves; (d) dead plant

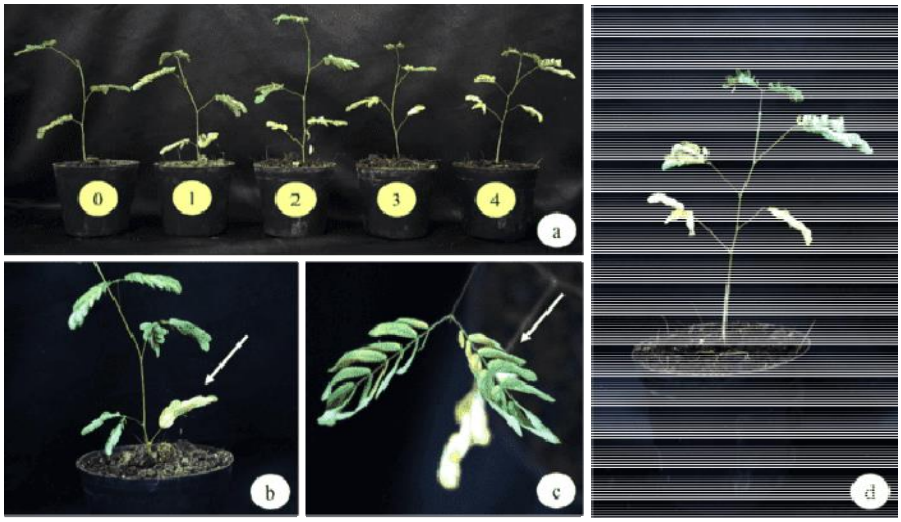

Figure5. Diseaseindexon*Leucaenaleucocephala*,(a)fromleft:healthyplant to100%wiltedleaves(score0−4);(b)initialsymptoms: yellowing from oldest leaves; (c) advanced symptoms: curved leaves; (d) yellowing upward

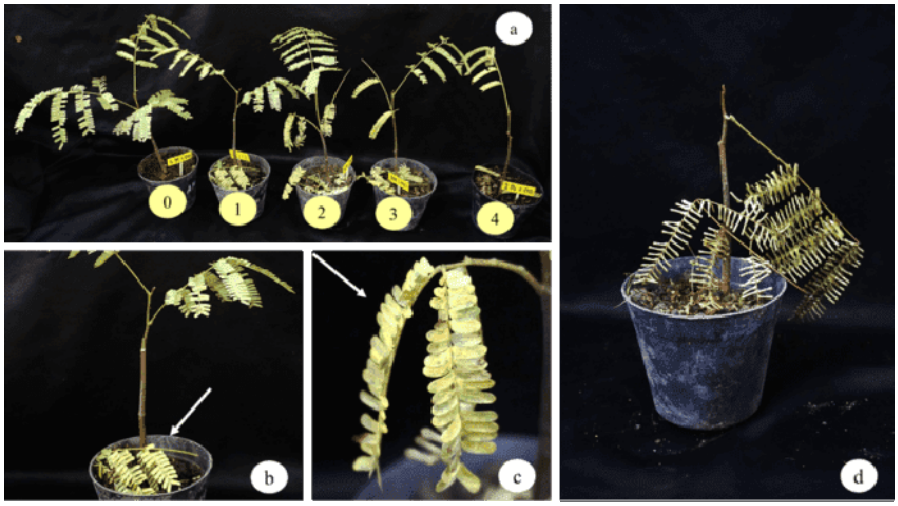

Figure6. Diseaseindexon*Parkiaspeciosa*,(a)fromleft:healthyplantto100%wiltedleaves(score0−4);(b)initialsymptoms: yellowing and dry from oldest leaves; (c) advanced symptoms: curved leaves; yellowing (d) dead plant

Figure7. Diseaseindexon*Acaciaauriculiformis*,fromleft:healthyplanttowiltedanddeadplant(score0–4)

RESULTSANDDISCUSSION

Pathogenicitytest

The results showed that all the six forest plants tested had similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of*F. oxysporum*infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progress to younger shoots, severe wilting, drying, falling of leaves, and eventually plant die. Another symptom that appeared was sudden wilting and death of plant without changing theleaf colour, while control plants did not show any symptoms (Figures 2-7).

Diseaseseveritywassignificantlyhigherthancontrols. *A.crassicarpa*and *F.moluccana*weremostseverely affectedwithanaveragescoreof4.00and3.44,respectively.Ontheotherhand, *A.pauciflorum*, *L. leucocephala*,and *P.speciosa*wereshowed moderate diseaseseverity i.e.1.96,1.68,and1.80,respectively, whereas*A.auriculiformis*hadthelowest(0.36)disease severity (Table 1).Based on the disease score, host plants were classified into three groups: i) highly susceptible (*A. crassicarpa* and*F. moluccana*), ii) moderately susceptible (*A. pauciflorum*P. *speciosa*, and*L. leucocephala*), and iii) moderateresistance/tolerance(*A.auriculiformis*).Result exhibited that there was no significant difference between thediseaseseverityinthesamehostthathadbeen inoculated with different isolates (Table 1).

Table1. Diseaseseverityandhostresponsesto*Fusariumoxysporum*isolatedfrom*Acaciamangium*

Plantspecies	Isolates[a)]					
	AF01[b)]	Response[c)]	BF05	Response	DF11	Response
Acaciacrassicarp	4.00a	HS[c)]	3.48	HS	3.96a	HS
Falcatariamoluccan	3.44ab	HS	3.04	HS	2.80ab	S
Archidendronpaucifloru	1.96b	MS	1.88b	MS	1.40cd	MS
Leucaenaleucocephal	1.52	MS	1.56b	MS	1.68bc	MS
Parkiaspecios	1.80	MS	1.04b	MS	2.16bc	S
Acaciaauriculiformi	0.36d	MR	0.40	MR	0.60d	MR

Valuesfollowedbythesameletterineachrowarenotsignificant.

[a]DI 0–4, where 0 = no disease/healthy seedling, 1 = yellow leaves, 2 = yellow leaves and slightly wilted, 3 = severe wilt, and 4 = dead seedling.

[b)]*F.oxysporum*isolates.

[c)] Host response grouped as: R = resistant (DI = 0); MR = moderately resistant/tolerance (DI = <1); MS = moderately susceptible (DI = 1.0–2.0); S = susceptible (DI = 2.1–3.0); HS = highly susceptible (DI = 3.1–4.0) (Bertetti et al. 2018).

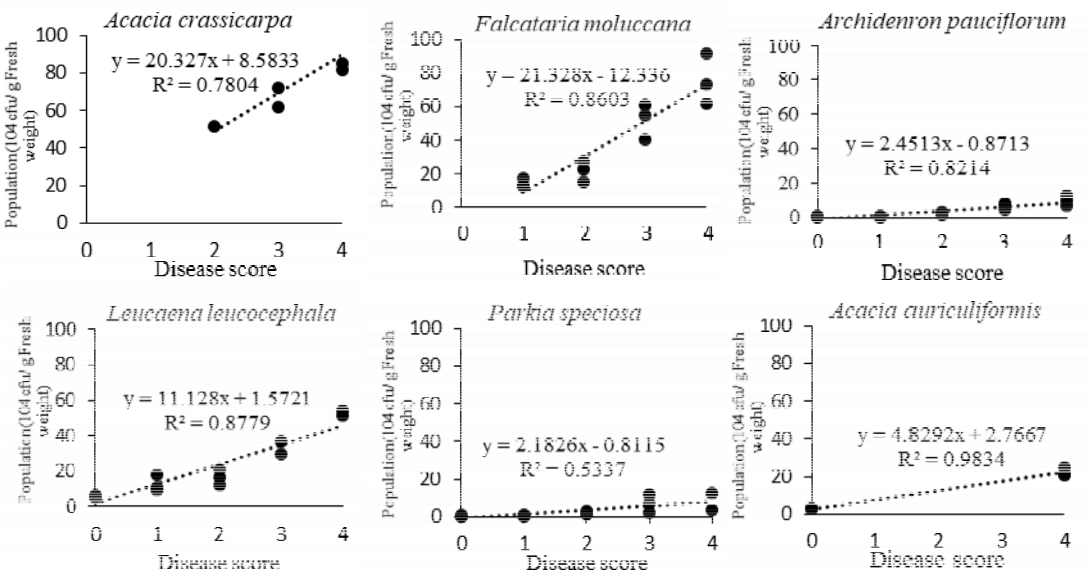

Figure8. Regressionanalysisofdiseasescorerateand*F.oxysporum*population

Table2.*Fusariumoxysporum*populationonrootineachdiseaseindex

Plantspecies	Populationof*Fusariumoxysporum*(×10^4CFU/gfreshweight)[a]					Average[c]
	0[b]	1	2	3	4	
AF01[d]						
Acaciacrassicarpa	n s	n.s	n.s	n.s	8513 a[e]	8513
Falcatariamoluccan	n.	1777	2277 a	6098 a	9187 a	7650
Archidendronpaucifloru	045b	110b	322b	815b	1253 cd	5 06
Leucaenaleucocephal	617a	1810 a	2093 a	n.s	5167b	2213
Parkiaspecios	032b	045b	258b	727b	357d	2 16
Acaciaauriculiformi	292a	n.	n.	n.	2453 c	4 65
BF05						
Acaciacrassicarp	n.	n.	5180 a	7208 a	10510	9261
Falcatariamoluccan	n.	1322	1532b	4033b	6167b	4385
Archidendronpaucifloru	047c	063b	173c	688c	990d	3 60
Leucaenaleucocephal	467a	902a	1232b	2932b	n.	1116
Parkiaspecios	0 48	057b	1 27	233d	n.	0 87
Acaciaauriculiformis	255b	n.s	n s	n.s	2043 c	3 98
DF11						
Acaciacrassicarp	n.	n.	n.	6192 a	8200 a	8120
Falcatariamoluccan	n.s	1250 a	2747 a	5493 a	7300 a	4793
Archidendronpaucifloru	0 35	035b	3 37	4 42	6 92	2 19
Leucaenaleucocephal	558a	1117	1653b	3663b	5427b	1969
Parkiaspecios	025c	048b	158c	1197d	1227d	5 79
Acaciaauriculiformi	283b	n.	n.	n.	2128 c	5 05

n.s:Nosample,cfu:colonyformingunit

[a]*F.oxysporum*populationcalculatedattheendoftheexperiment(21daysafterinoculation).

[b]DI0–4;0=nodisease/healthyseedling;1=yellowleaves;2=yellowleavesandslightlywilted;3=severewilt;and4=deadseedling.

[c]Averageof*F.oxysporum*population(cfu/gfreshweight)=($P_0A+P_1B+P_2C+P_3D+P_4E$)/N;wherePO,P1,P2,P3,andP4=populationof pathogeninscore0, 1,2,3,and4:A=number of plantson score0; B =number of plantson score1; C =number ofplantson score2; D =number ofplantson score3; E = number ofplantson score4; N = totalnumber of plants.

[d]*F.oxysporum*isolates

[e]Valuesfollowedbythesameletterineachrowarenotsignificant.

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Table3.*Fusariumoxysporum*populationaverageanddiseasesindexofplant

Plantspecies	Populationaverage($\times 10^4$CFU/gfresh weight)[a]			Diseaseindex[b]		
	AF01[c]	BF05	DF11	AF01	BF05	DF11
Acaciacrassicarp	8513	9261	8120	4.00	3.48	3.96
Falcatariamoluccan	7650	4385	4793	3.44	3.04	2.80
Archidendronpaucifloru	5 06	3.60	2.19	1.96	1.88	1.40
Leucaenaleucocephal	2213	1116	1969	1.52	1.56	1.68
Parkiaspecios	2 16	0.87	5.79	1.80	1.04	2.16
Acaciaauriculiformi	4 65	3.98	5.05	0.36	0.40	0.60

[a]Average of*F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4 = population ofpathogenin score0,1,2,3,and4: A=numberofplantson score 0;B= numberofplantsonscore 1;C= numberofplantsonscore 2; D = number of plants on score 3; E = number of plants on score; N = total number of plants.
[b]DI 0–4; 0 = no disease/healthy seedling; 1 = yellow leaves; 2 = yellow leaves and slightly wilted; 3 = severe wilt; and 4 = dead seedling.
[c]*F.oxysporum*isolates.

Discussion

Arecentstudyreportedanextraordinaryincidenceof seedlingwiltdiseasecausedbyfungalpathogen *F. oxysporum* attacking commercial nurseries of*A. mangium* inSouthSumatra(Solehaetal.2021).Therefore,the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Hostrangetestsalsoprovideinformationabout plant species that have the potential to become alternative hosts or main hosts for the pathogen (Sampaio et al. 2021).

Theresults indicated that*F. oxysporum*,which causes vascularwiltin*A.mangium*nursery,canalsoinfect fabaceae plantswith varioushostresponses.*A. crassicarpa* and *F.moluccana*werehighlysusceptible,while *A. pauciflorum*, *L.leucocephala*,and *P.speciosa*were moderately vulnerable, and *A. auriculiformis* was moderatelyresistant.Pathogencausedwiltingsymptoms in all test plant species with DI of 4.00. Although DI was lower (0.36) in*A. auriculiformis*, but it had the potential to damageplants. *F.oxysporum*abletoinfectplantseven withalowDI,causingthedeathofcultivars.Moreover, when a plant is grown in contaminated soil, there is a high risk ofdamageto crops. Asimilar incidentwasreportedby Pastranaetal.(2017)inwhich *F.oxysporum*from blackberryalsocausedsuddendeathinstrawberries. Another study also revealed that*F. oxysporum* from cactus causes root and stem rot diseasesin*Euphorbia* (Bertetti et al. 2017).

The results revealed that several types of plants belonging to the fabaceae family had great potential to become an alternative hosts and even main host for *F. oxysporum* when planted in the same field. Widespread of this pathogen may allow interaction with new plants (Edel-Hermann and Lecomte 2019; Sampaio et al. 2021). Moreover, the planting of new species affected the occurrenceofnewoutbreaksbecausethepathogenicstrains adapted to thesoil and had became virulent (Sampaio et al. 2021; Stukenbrock and McDonald 2008). Furthermore, nurseryactivitiesthatusecontaminatedsoilrepeatedlyalso triggered the pathogens proliferation and adaptation toother plants.

Thepathogenpopulationin *A.crassicarpa*and *F. moluccana*grewveryrapidlywithincreasingdisease scores,whilein*L.leucocephala*grewmoderately, and *A. pauciflorum*, *P.speciose*,and *A.auriculiformis*grew slowly.Inthisstudy,thepopulationof*F.oxysporum*on highly susceptible plants (*A. crassicarpa* and *F. moluccana*)wassignificantlyhigherthanotherplantsfor eachdiseasescore.Thispatterniscommonwherethe populationofpathogenisalsohigherwithdiseasescores (Scottetal.2014).deBorbaetal.(2017)reportedthat susceptiblelettucecultivarsshowedhigh *Fusarium* populationlevelandvulnerableblackbeangenotype showedapopulationlevelof15.4×10^5CFU^{g-1}.The second patternwasobservedon*L. leucocephala*, where the population of pathogen was also moderate with a moderate diseasesscore.Thesimilarresultwasalsooccurredin garlic with a disease severity of 44% due to*Fusarium* spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

Aspecialpatternoccurredon*A.pauciflorum*inwhich *F.oxysporum*causedamoderateinfection,butthe pathogenpopulationwaslow.Thismightbeduetothe plant defence mechanism. Scott et al. (2014)reported that resistant pepper plants also support pathogen development inroots,evenwithoutexternalsymptoms.Similarphenomenaw asreportedbyMuslimetal.(2003a)who notedthatsometomatoplantsareinfectedmoderately (score1–2)by *F.oxysporum*f.sp. *lycopersici*,butthe population was lower than other plants in same score.

The infection and total population on*Parkia speciosa* and*A. auriculiformis* was lower. Thisindicated thatplants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with*F. oxysporum* f. sp.*fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonisation and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited tothe epidermis, then the pathogens do notreach the vascular tissue. Van Den Berg et al. (2007) reported that banana clones tolerant to *F. oxysporum* f. sp. *cubense* correspond withthis,with asignificantincrease intheinductionof cell

wall-associated phenolic compounds. Jiménez-Fernándezet al. (2013) also reported that*Fusarium oxysporum* f. sp. *ciceris* race 0 remained in the intercellular space of root cortex and failed to reach xylem in resistant chickpea cultivars.

Inthisstudy, *A.crassicarpa*and *F.moluccana* were proven to be an alternative host of*F.oxysporum.* Whereas *L.leucocephala*, *A.pauciflorum*, *P.speciosa*,and *A. auriculiformis*hadpotentialasalternativehosts.Many plantsoffabaceae familywasattackedbyformaespecialis *F.oxysporum*,suchas*Vignaangularis*(*F.oxysporum*f.sp. *adzukicola*),*Cicerarietinum*,*Cicer*spp.(*F.oxysporum*f. sp. *ciceris*), *Acacia*spp.(*F.oxysporum* f.sp. *koae*), *Lens culinaris*,*L.esculenta*(*F.oxysporum* f.sp. *lentis*), *Medicagosativa*(*F.oxysporum* f.sp. *medicaginis*), *Phaseolusvulgaris*, *P.coccineus*(*F.oxysporum* f.sp. *phaseoli*),*Pisumsativum*,*Cicerarietinum*(*F.oxysporum*f. sp.*pisi*) (Edel-Hermann and Lecomte 2019). However, in this study,*F. oxysporum* isolatedfrom *A.mangium* has a wide host range fromfabaceae family; therefore, it is not classified as formae specialis.

In conclusion,*F. oxysporum*isolated from*A. mangium* causes infection in several types of forest and industrial plants.Since it has a wide host range, it is not classified as part of the formae specialis group.

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To: SOLEHA SOLEHA <soleha057@gmail.com>, AHMAD MUSLIM <a_muslim@unsri.ac.id>, SUWANDI SUWANDI <Suwandi@fp.unsri.ac.id>,SABARUDDINKADIR<sabar@pps.unsri.ac.id>,RAHMATPRATAMA <rahmatpratama@pps.unsri.ac.id>

SOLEHASOLEHA,AHMADMUSLIM,SUWANDISUWANDI,SABARUDDINKADIR,RAHMATPRATAMA:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Host range studies of Fusarium oxysporum, causal agent of seedling wilt disease of Acacia mangium".

Ourdecisionisto:AcceptSubmission

BestRegards,
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BiodiversitasJournalofBiologicalDiversity

a. muslim unsri <a_muslim@unsri.ac.id> Thu, Dec 16, 2021 at 11:44 AM
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DearEditorinChief

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ThankyouverymuchforyourquickresponseregardingAcceptanceLetterforourmanuscripts

WearesohappytoreceiveAcceptanceLetterof ourmanuscriptentitled"Hostrangestudiesof Fusarium oxysporum, causal agent of seedling wilt disease of Acacia mangium"

ThankyouverymuchforyourkindnessandexcellentcooperationSincerely,
AhmadMuslim
Senior lecturer
FacultyofAgriculture,SriwijayaUniversity
Jl.Palembang-PrabumulihKm.32,Indralaya,Palembang,Indonesia E-mail: a_muslim@unsri.ac.id

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3. Bukti konfirmasi artikel untuk di published online

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The editing of your submission, "Host range studies of Fusarium oxysporum, causal agent of seedling wilt disease of Acacia mangium," is complete. We are now sending it to production.

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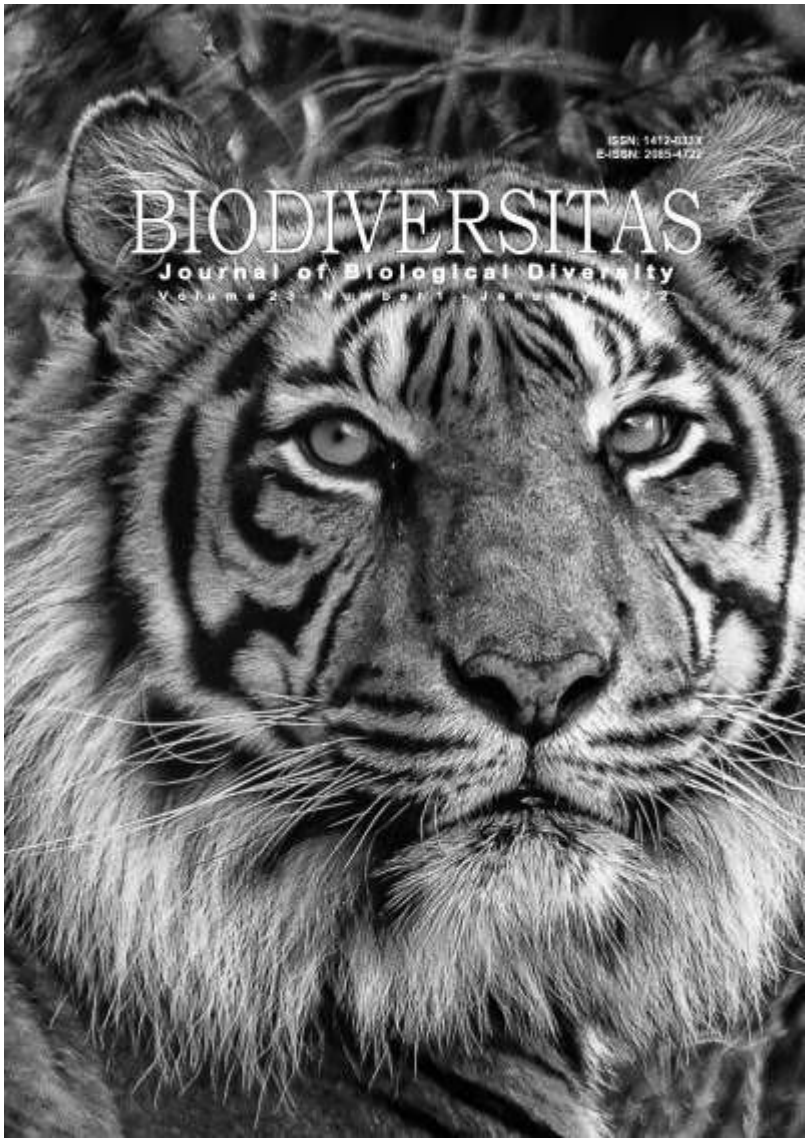

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SOLEHASOLEHA
Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl Raya Palembang-PrabumulihKm32,Indralaya,OganIlir30662,SouthSumatra,Indonesia

AHMADMUSLIM♥(MAILTO:A_MUSLIM@UNSRI.AC.ID)
Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. Jl Raya Palembang-PrabumulihKm32,Indralaya,OganIlir30662,SouthSumatra,Indonesia

SUWANDISUWANDI
Department of Plant Protection, Faculty of Agriculture, Universitas Sriwijaya. Jl Raya Palembang-PrabumulihKm32,Indralaya,OganIlir30662,SouthSumatra,Indonesia

SABARUDDINKADIR
DepartmentofSoilSciences,FacultyofAgriculture,UniversitasSriwijaya.JlRayaPalembang-PrabumulihKm32,Indralaya,OganIlir30662,SouthSumatra,Indonesia

RAHMATPRATAMA
Program of Agriculture Sciences, Faculty of Agriculture, Universitas Sriwijaya. Jl Raya Palembang-PrabumulihKm32,Indralaya,OganIlir30662,SouthSumatra,Indonesia

Abstract

Abstract.*SolehaS,MuslimA,SuwandiS,KadirS,PratamaR.2021.Hostrangestudiesof*Fusarium oxysporum, *causal agent of seedling wilt disease of* Acacia mangium. *Biodiversitas 23: 25-32.Fusariumoxysporum*isaseriouspathogenthatcausesseverewiltdiseasein commercialnurseriesof*Acaciamangium*inSouthSumatra,Indonesia.Thisstudyaimedto investigatethehostrangeof*F.oxysporum*asanurserywiltpathogenin*A.mangium*and severalforestandindustrialplants.Threeisolatesof*F.oxysporum*withdifferenttranslation elongation factor (*tef* 1-a) sequences were tested for pathogenicity on different fabaceae familyplantsandthegrowthofpopulationwasalsoobserved.Theresultsshowedthatallthe three isolates were able to infect all the tested plants with different reactions of wilt disease. The *Acacia crassicarpa* and *Falcataria moluccana* were highly susceptible; *Archidendron pauci*⁄*rum*,*Leucaenaleucocephala*,and*Parkiaspeciosa*weremoderatelyvulnerableand*Acacia auriculiformis*wasmoderatelyresistant.Thepathogenpopulationin*A.crassicarpa*and*F. moluccana*grewrapidlyalongwiththeincreaseindiseasescores,whilein*L.leucocephala*it was moderate, and slow in *A.pauci*⁄*rum*, *P.speciosa*and *A.auriculiformis*plants. In conclusion, *F.oxysporum*pathogen,whichwasisolatedfrom*A.mangium*,hasawiderangeofhostsinthe fabaceaefamily.

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Hostrangestudiesof*Fusariumoxysporum*,causalagentofseedlingwilt disease of *Acacia mangium*

SOLEHASOLEHA[1],AHMADMUSLIM[2,♥],SUWANDISUWANDI[2],SABARUDDINKADIR[3], RAHMAT PRATAMA[1]

[1]ProgramofAgricultureSciences,FacultyofAgriculture,UniversitasSriwijaya.JlRayaPalembang-PrabumulihKm32,Indralaya,OganIlir30662,South Sumatra, Indonesia
[2]DepartmentofPlantProtection,FacultyofAgriculture,UniversitasSriwijaya.JlRayaPalembang-PrabumulihKm32,Indralaya,OganIlir30662,SouthSumatra, Indonesia. Tel./fax.: +62-896-3874-9695, ♥email: a_muslim@unsri.ac.id
[3]DepartmentofSoilSciences,FacultyofAgriculture,UniversitasSriwijaya.JlRayaPalembang-PrabumulihKm32,Indralaya,OganIlir30662,SouthSumatra, Indonesia

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Abstract.*SolehaS,MuslimA,SuwandiS,KadirS,PratamaR.2021.Hostrangestudiesof*Fusariumoxysporum,*causalagentof seedling wilt disease of*Acacia mangium*. Biodiversitas 23: 25-32.Fusarium oxysporum*is a serious pathogen that causes severe wilt disease in commercial nurseries of*Acacia mangium*in South Sumatra, Indonesia. This study aimed to investigate the host range of*F. oxysporum*asanurserywiltpathogenin*A.mangium*andseveralforestsandindustrialplants.Threeisolatesof*F.oxysporum*with differenttranslationelongationfactor(*tef*1-α)sequenceswereretestedforpathogenicityondifferentFabaceaefamilyplantsandthe growth of population was also observed. The results showed that all three isolates were able to infect all the tested plants with different reactions to wilt disease. *Acacia crassicarpa* and*Falcataria moluccana* were highly susceptible;*Archidendron pauciflorum*, *Leucaena leucocephala*,and *Parkiaspeciosa* weremoderatelyvulnerableand *Acaciaauriculiformis*wasmoderatelyresistant.Thepathogen population in *A. crassicarpa* and *F. moluccana* grew rapidly along with the increase in disease scores, while in *L. leucocephala* it was moderate,andslowin *A.pauciflorum*, *P.speciosa*and *A.auriculiformis* plants.Inconclusion, *F.oxysporum* pathogen,whichwas isolated from *A. mangium*, has a wide range of hosts in the Fabaceae family.

Keywords:*Acaciamangium*,Fabaceae,*Fusariumoxysporum*,hostrange,seedlingwilt

INTRODUCTION

Acacia mangium (Willd.) is a species of plant that originated in several regions of Indonesia, Papua New Guinea,andAustralia,andwhich,hasalsobeenfoundfora few decades in the humid tropical lowlands of Asia, South America, and Africa (Koutika and Richardson 2019). It is planted on a large scale for industrial purposes and forest restoration in the tropics (Matsumura and Naoto 2011). Since this plant species is known for its fast growth and highadaptabilitytovariousenvironmentalconditions (Asif etal. 2017), itiswidely used for agroforestry, forestry, and restoration of degraded land (Koutika and Richardson 2019).

*Fusariumoxysporum*isanimportantpathogenicfungus that causes wilt disease in different plants all over the world.Soleha et al. (2021) reported that it was identifiedas the causative agent of vascular wilt in several commercial nurseries of *A. mangium* in South Sumatra.The main source of transmission is through infected seedlings and soil, which is relatively difficult to treat after contamination. The fungus survives by forming chlamydospores that allow it to live for a long time, even without a host plant (Ignjatov et al. 2012; Koyyappurath et al. 2016; Rana et al. 2017; Muslim et al. 2019). Furthermore, it attacks almost every type of plant, from cultivatedtoforestandwild(e.g.weeds)(Joshi2018).This fungusisalsoabletoattackvariousplanthabitssuchas trees(Zhangetal.2013),herbaceousplants(Jacobsand Heerden 2012), and vines (Rooney-Latham and Blomquist 2011).Severaltypesofforestplantsthathavereportedly been attacked by*F. oxysporum* are*Pinus massoniana*(Luo andYu2020), *Tectonagrandis* (Borgesetal.2018), *Pseudotsugamenziesii* (Stewartetal.2011), *Acacia mangium* (Widyastuti et al. 2013), and others.

Since *F. oxysporum*has a high level of host specificity, it is classified as a formae species (Burkhardt et al. 2019; Tayloretal.2019).AccordingtoLeslieandSummerell (2006) more than 100 formae species and races have been identified and are widespread in the world.

Besides *A.mangium*,whichisthemainplantof industrialforestryinIndonesia,otherplants,suchas*Acacia crassicarpa*, *Acaciaauriculiformis*, *Parkiaspeciosa*, *Archidendronpauciflorum*, *Falcataria moluccana*,and *Leucaenaleucocephala*arealsoimportantandhavehigh economic value. Considering that they belong to the same family(Fabaceae),theycanbecomethemainoralternative hostsfor *F.oxysporum*, causativeagentofwiltdisease. Thisstudyaimedtoinvestigatethehostrangeof *F.oxysporum* as a nursery wilt pathogenin*A. mangium* and several industrial and local forest plants in Indonesia.

MATERIALSANDMETHODS

Fungalisolates

Threepathogenicisolatesof *F.oxysporum*(AF01, BF05, and DF11) were selected, which were differentiated accordingtotheir *tef*1-αsequence(Figure1).Isolates wereculturedonPDBliquidmedium(potatodextrose broth) and incubated at 26-28°C on a shaker (150 rpm) for about five days. Then the mycelia suspension was filtered usingtwolayersofsterilegauzetoseparatetheconidiaand hyphae.Theconidialconcentrationwasdetermined usinga hemocytometer and then adjusted to a concentration of 10^6 ml^{-1} for pathogenicity test.

Plantmaterial

The plants used were members of the Fabaceae family, namely*A. crassicarpa, A. auriculiformis,F. moluccana,A. pauciflorum,P. speciosa*, and*L. leucocephala*, which were one month old. The seedlings were obtained from theForest Crops Research Institute, South Sumatra. Seedlings were transferred in a mixed medium with cocopeat (1:1) usingaplasticpotof10cmdiameterand10cmheight,and then placed in a shade house.

Pathogenicitytest

A pathogenicity test was carried out using root dip method, in which the roots were washed under running water and then immersed in 250 ml of conidia suspension (10^6 conidia ml^{-1}) for 15 minutes. The control plants were immersed in sterile distilled water, and the seedlings were transplanted into plastic pots and placed under a house shade. Each isolate was inoculated on 25 plants with five replicates (five plants per-replicate). Then, disease severity wascalculatedusingthemethodofMuslimetal.(2003a) and the disease index(DI) was classified into following grades, where 0 : no disease/healthy seedling, 1 : yellow leaves,2:yellowleavesandslightlywilted,3 :severewilt, and 4 : dead seedling (Bertetti et al. 2018). Furthermore, plantresponsesweregroupedas,R :resistant(DI=0),MR: moderately resistant/tolerance (DI = <1), MS : moderately susceptible (DI = 1.0–2.0), S : susceptible (DI = 2.1–3.0) and HS : highly susceptible (DI = 3.1–4.0). The development of disease was observed 1–21 days after inoculation.

*Fusariumoxysporum*population

Thepopulationof *F.oxysporum* intherootswas calculated attheend ofthe experimentusingthemethod of (Muslimetal.2003b;Lietal.2009;Horinouchietal. 2011)withmodificationstothesurfacesterilizationof samples.Thentheplantsweregroupedaccordingto severity(diseasescore)andwashedseparatelyunder running water to remove soil residues. After that, all plants ineachscoreweresurfacesterilizedusing1%sodium hypochloritefor15minutes,thenrinsedthreetimeswith distilledwater.Thesamplesandwater(1:100w/v)were homogenizedusingblenderat8000rpmfor10minutes. Thentheywerefilteredusingtwolayersofsterilegauze anddiluted 10 to1000 times. The suspension was spread onPeptonePCNBagarMedia(PPA/NashSnyderMedium) (Leslie and Summerell 2006) in triplicate (five Petri dishes perreplication)andincubatedindarkforsevendaysat roomtemperature.Thenumberofcolony-formingunits (CFU) of *F. oxysporum*was calculated on the basis of fresh weightpergramofsampleandgroupedaccordingtothe level of diseases severity.

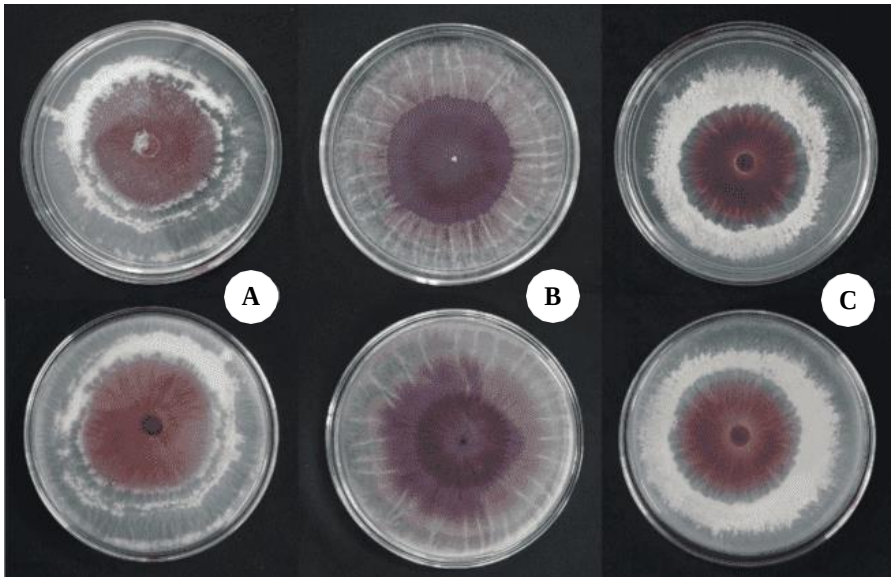

Figure 1.*Fusarium oxysporum* isolates on PDA medium. A. AF01, B. BF05, and C. DF11. First line: front view; second line: reverse view

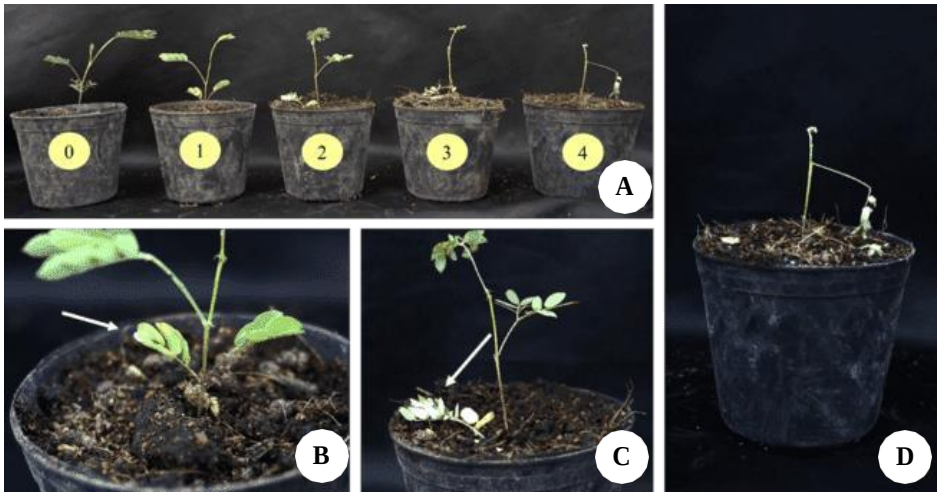

Figure 2. Disease index of*Acaciacrassicarpa*,A.From left: healthy plant to100%wiltedleaves (score 0−4);B. Initial symptoms: yellowing from oldest leaves; C. Advanced symptoms: falling leaves; D. Dead plant

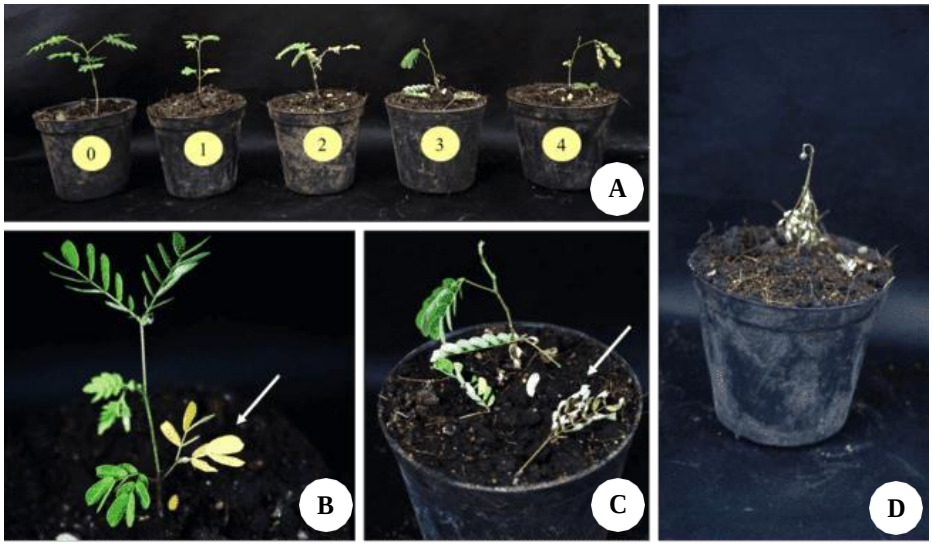

Figure 3. Disease index on*Falcataria moluccana,* A. From left: healthy plant to 100% wilted leaves (score 0−4); B. Initial symptoms: yellowing from oldest leaves; C. Advanced symptoms: curved, dry, and falling leaves; D. Dead plant

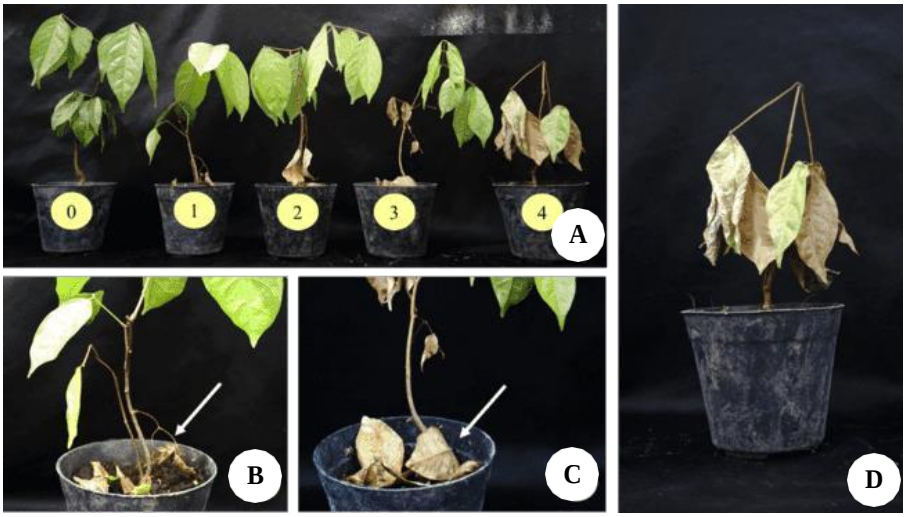

Figure4. Diseaseindexon*Archidendronpauciflorum*,A.Fromleft:healthyplantto100%wiltedleaves(score0−4);B.Initial symptoms: yellowing and dry from oldest leaves; C. Advanced symptoms: falling leaves; D. Dead plant

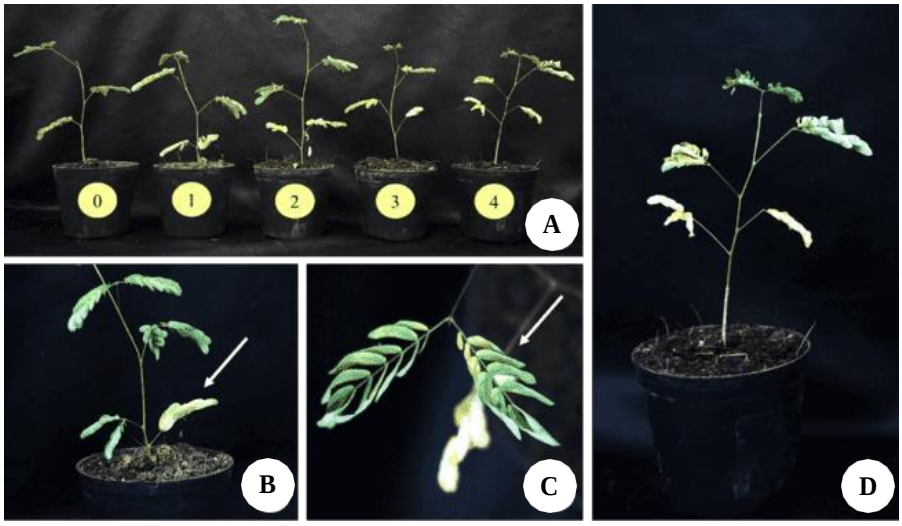

Figure5. Diseaseindexon*Leucaenaleucocephala*,A.Fromleft:healthyplantto100%wiltedleaves(score0−4);B.Initialsymptoms: yellowing from oldest leaves; C. Advanced symptoms: curved leaves; D. Yellowing upward

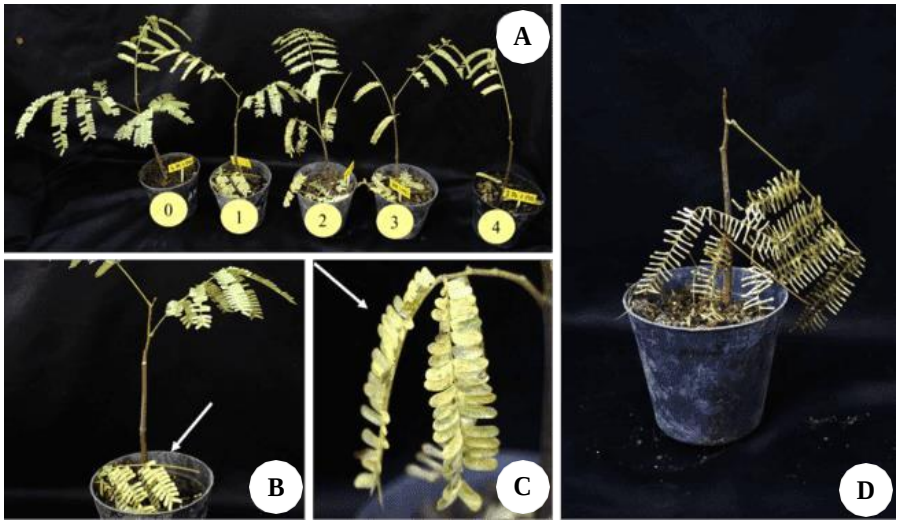

Figure6. Diseaseindexon*Parkiaspeciosa*.A.Fromleft:healthyplantto100%wiltedleaves(score0−4);B.Initialsymptoms: yellowing and dry from oldest leaves; C. Advanced symptoms: curved leaves; yellowing, D. Dead plant

Figure7. Diseaseindexon*Acaciaauriculiformis*,fromleft:healthyplanttowiltedanddeadplant(score0–4)

RESULTSANDDISCUSSION

Pathogenicitytest

The results showed that all the six forest plants tested had a similar reaction to the pathogen. Seven days after inoculation, all the plants showed typical symptoms of*F. oxysporum*infection, i.e. yellowing of oldest leaves closest to the stem base, which gradually progresses to younger shoots, severe wilting, drying, falling of leaves, and eventually plant die. Another symptom that appeared was suddenwiltinganddeathofplant withoutchangingtheleaf color, while control plants did not show any symptoms (Figures 2-7).

Diseaseseveritywassignificantlyhigherthancontrols. *A.crassicarpa*and *F.moluccana*weremostseverely affectedwithanaveragescoreof4.00and3.44,respectively.Ont heotherhand, *A.pauciflorum*, *L. leucocephala*,and *P.speciosa*wereshowedmoderate diseaseseverity,i.e.1.96,1.68,and1.80,respectively, whereas*A.auriculiformis*hadthelowest(0.36)disease severity (Table 1).Based on the disease score, host plants were classified into three groups: (i) highly susceptible (*A. crassicarpa* and*F. moluccana*), (ii) moderately susceptible (*A. pauciflorumP. speciosa*, and*L. leucocephala*), and(iii) moderateresistance/tolerance(*A.auriculiformis*).Results exhibited that there was no significant difference between thediseaseseverityinthesamehostthathadbeen inoculated with different isolates (Table 1).

*Fusariumoxysporum*population

The total population of*F. oxysporum* on the roots was determined by calculating the CFU for each category of damage.ForDI4, *A.crassicarpa*and *F.moluccana* showed a significantly higher population (82.00–105.10 × 10^4CFUg^{-1}freshweight)thanotherplants.Thelowest population was recorded in *P. speciosa*and *A. pauciflorum* (3.57–12.27×10^4CFUg^{-1}freshweight).Thissamepattern also occurred in DI 2 and 3, while no sample was recorded in*A.auriculiformis*forDI2and3.InDI1,thehighest populationwasrecordedin *F.moluccana*and *L.leucocephala*,while *A.crassicarpa*and *A.auriculiformis* had no sample for DI 1. In inoculated plants with DI 0, the populationwassignificantlyhigherin*L.leucocephala*and *A.auriculiformis*andnosamplewasnotedin*A. crassicarpa* and *F. moluccana* (Table 2 and Table 3).

Theregressionanalysisresultsshowedthatallplants except*P.speciosa*hadalinearrelationshippatternbetween theincreaseindiseasescoreandpopulation.Thepathogenic population on*A. crassicarpa* and*F. moluccana* grew rapidly along with the increase in disease scores, as indicatedbythemagnitudeofregressiongradient coefficient(m=20.3–21.3).However,moderateincrease wasobservedin*L.leucocephala*(m=11.2)(m=11.2)and veryslowin *A.pauciflorum*, *P.speciose*,and *A. auriculiformis* (m=2.2–4.8) (Figure 8).

Table 3 showed that isolates were different in*tef1-α*,but the population and DI patterns were similar for eachtest plant. The correlation between the population of pathogen (g^{-1} fresh weight) and the level of DI was described as follows: i) high pathogen populations with high DI (*A. crassicarpa* and*F. moluccana*), ii) moderate population with moderate DI (*L. leucocephala*), iii) low population with moderate DI (*A. pauciflorum*), and iv) low populationwithlowDI(*P.speciosa*and*A.auriculiformis*).

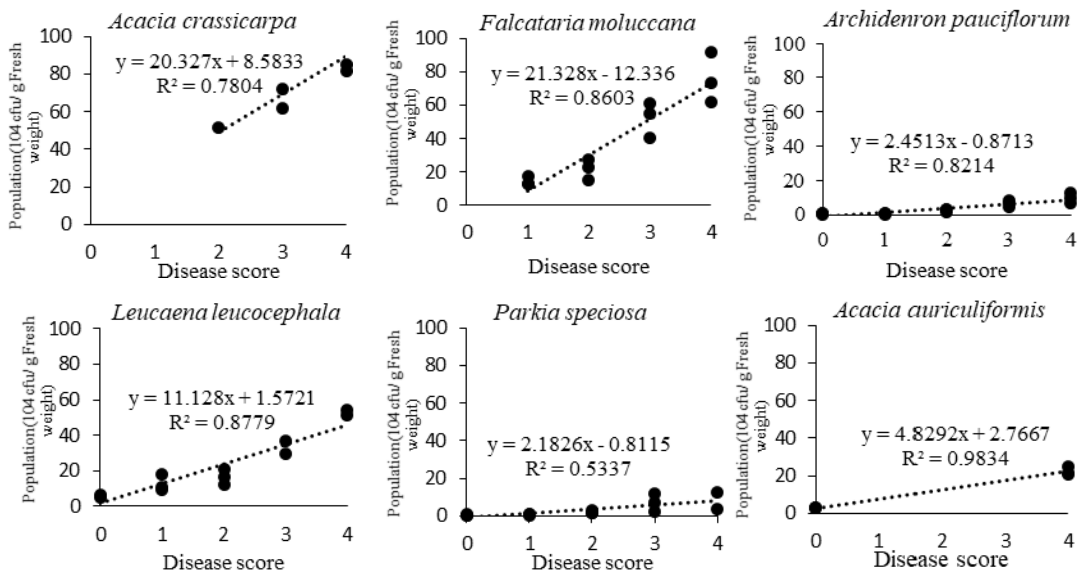

Figure8. Regressionanalysisofdiseasescorerateand*Fusariumoxysporum*population

Table1. Diseaseseverityandhostresponsesto*Fusariumoxysporum*isolatedfrom*Acaciamangium*

Plantspecies	Isolates[a)]					
	AF01[b)]Re	sponse[c)]BF05Re		sponseDF11Re		sponse
Acaciacrassicarpa	4.00a	HS[c)]	3.48a	HS	3.96a	HS
Falcatariamoluccana	3.44ab	HS	3.04a	HS	2.80ab	S
Archidendronpauciflorum	1.96bc	MS	1.88b	MS	1.40cd	MS
Leucaenaleucocephala	1.52c	MS	1.56b	MS	1.68bc	MS
Parkiaspeciosa	1.80c	MS	1.04bc	MS	2.16bc	S
Acaciaauriculiformis	0.36d	MR	0.40c	MR	0.60d	MR

Note: Values followed by the same letter in each row are not significant.[a] DI 0–4, where 0: no disease/healthy seedling, 1: yellow leaves,2:yellowleavesandslightlywilted,3:severewilt,and4:deadseedling. [b)]*F.oxysporum*isolates. [c)]Hostresponsegroupedas:R: resistant (DI = 0); MR: moderately resistant/tolerance (DI = <1); MS: moderately susceptible (DI = 1.0–2.0); S: susceptible (DI = 2.1–3.0); HS: highly susceptible (DI = 3.1–4.0) (Bertetti et al. 2018).

Table2.*Fusariumoxysporum*populationonrootineachdiseaseindex

Plantspecies	Populationof*Fusariumoxysporum*(×10^{4}CFU/gfreshweight)[a)]					Average[c)]
	0[b)]	1	2	3	4	
AF01[d)]						
Acaciacrassicarpa	n.s	n.s	n.s	n.s	85.13a[e)]	85.13
Falcatariamoluccana	n.s	17.77a	22.77a	60.98a	91.87a	76.50
Archidendronpauciflorum	0.45b	1.10b	3.22b	8.15b	12.53cd	5.06
Leucaenaleucocephala	6.17a	18.10a	20.93a	n.s	51.67b	22.13
Parkiaspeciosa	0.32b	0.45b	2.58b	7.27b	3.57d	2.16
Acaciaauriculiformis	2.92a	n.s	n.s	n.s	24.53c	4.65
BF05						
Acaciacrassicarpa	n.s	n.s	51.80a	72.08a	105.10a	92.61
Falcatariamoluccana	n.s	13.22a	15.32b	40.33b	61.67b	43.85
Archidendronpauciflorum	0.47c	0.63b	1.73c	6.88c	9.90d	3.60
Leucaenaleucocephala	4.67a	9.02a	12.32b	29.32b	n.s	11.16
Parkiaspeciosa	0.48c	0.57b	1.27c	2.33d	n.s	0.87
Acaciaauriculiformis	2.55b	n.s	n.s	n.s	20.43c	3.98
DF11						
Acaciacrassicarpa	n.s	n.s	n.s	61.92a	82.00a	81.20
Falcatariamoluccana	n.s	12.50a	27.47a	54.93a	73.00a	47.93
Archidendronpauciflorum	0.35c	0.35b	3.37c	4.42c	6.92e	2.19
Leucaenaleucocephala	5.58a	11.17a	16.53b	36.63b	54.27b	19.69
Parkiaspeciosa	0.25c	0.48b	1.58c	11.97d	12.27d	5.79
Acaciaauriculiformis	2.83b	n.s	n.s	n.s	21.28c	5.05

Note: n.s: No sample, cfu: colony-forming unit. [a)]*F. oxysporum* population calculated at the end of the experiment (21 days after inoculation). [b)]DI 0–4; 0: no disease/healthy seedling; 1: yellow leaves; 2: yellow leaves and slightly wilted; 3: severe wilt; and 4: dead seedling. [c)] Average of*F. oxysporum* population (cfu/g fresh weight) = ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4: population ofpathogen in score0,1,2,3, and4:A:numberofplantson score0;B:numberofplantson score1;C:numberofplantson score 2; D: number of plants on score 3; E: number of plants on score 4; N: total number of plants. [d)]*F. oxysporum*isolates.[e)] Values followed by the same letter in each row are not significant.

Table3.*Fusariumoxysporum*populationaverageanddiseasesindexofplant

Plantspecies	Populationaverage(×10^{4}CFU/gfreshweight)[a)]			Diseaseindex[b)]		
	AF01[c)]	BF05	DF11	AF01	BF05	DF11
Acaciacrassicarpa	85.13	92.61	81.20	4.00	3.48	3.96
Falcatariamoluccana	76.50	43.85	47.93	3.44	3.04	2.80
Archidendronpauciflorum	5.06	3.60	2.19	1.96	1.88	1.40
Leucaenaleucocephala	22.13	11.16	19.69	1.52	1.56	1.68
Parkiaspeciosa	2.16	0.87	5.79	1.80	1.04	2.16
Acaciaauriculiformis	4.65	3.98	5.05	0.36	0.40	0.60

Note: [a)] Average of*F. oxysporum* population (cfu/g fresh weight): ($P_0A+P_1B+P_2C+P_3D+P_4E$)/N; where P0, P1, P2, P3, and P4: population ofpathogen in score0,1,2,3, and4:A:numberofplantson score0;B:numberofplantson score1;C:numberofplantson score 2; D: number of plants on score 3; E: number of plants on score; N: total number of plants. [b)] DI 0–4; 0: no disease/healthy seedling; 1: yellow leaves; 2: yellow leaves and slightly wilted; 3: severe wilt; and 4: dead seedling. [c)]*F. oxysporum* isolates

Discussion

Arecentstudyreportedanextraordinaryincidenceof seedlingwiltdiseasecausedbyfungalpathogen *F. oxysporum* attacking commercial nurseries of*A. mangium* inSouthSumatra(Solehaetal.2021).Therefore,the investigation of a new host of the pathogen is an important step in the plant protection strategy for soil-borne diseases. Hostrangetestsalsoprovideinformationaboutplantspecies that have the potential to become alternative hosts or main hosts for the pathogen (Sampaio et al. 2021).

Theresults indicated that*F. oxysporum*,which causes vascularwiltin*A.mangium*nursery,canalsoinfect Fabaceae plants with various host responses. *A. crassicarpa*and *F.moluccana*werehighlysusceptible, while *A.pauciflorum, L.leucocephala,*and *P.speciosa* weremoderatelyvulnerable,and *A.auriculiformis*was moderatelyresistant.Pathogencausedwilting symptomsin alltestplantspecieswithDIof4.00.AlthoughDIwas lower (0.36) in*A. auriculiformis*, but it had the potential to damageplants.*Fusariumoxysporum*isabletoinfectplants evenwithalowDI,causingthedeathofcultivars. Moreover,whenaplantisgrownincontaminatedsoil, there is a high risk of damage to crops. A similar incident wasreportedbyPastranaetal.(2017)inwhich*F. oxysporum*fromblackberryalsocausedsuddendeathin strawberries. Anotherstudy also revealed that *F. oxysporum*fromcactuscausesrootandstemrot diseasesin *Euphorbia* (Bertetti et al. 2017).

Theresultsrevealedthatseveraltypesofplants belongingtotheFabaceaefamilyhadgreatpotentialto becomeanalternativehostandevenmainhostfor *F. oxysporum* when planted in the same field. Widespread of thispathogen may allow interaction with new plants (Edel-HermannandLecomte2019;Sampaioetal.2021). Moreover,theplantingofnewspeciesaffectedthe occurrenceofnewoutbreaksbecausethepathogenicstrains adapted to thesoilandhad becomevirulent (Sampaio etal. 2021;StukenbrockandMcDonald2008).Furthermore, nurseryactivitiesthatusecontaminatedsoilrepeatedlyalso triggered the proliferation and adaptation of the pathogens to other plants.

Thepathogenpopulationin *A.crassicarpa*and *F. moluccana*grewveryrapidlywithincreasingdisease scores,whilein*L.leucocephala*grewmoderately, and *A. pauciflorum, P.speciose*,and *A.auriculiformis*grew slowly.Inthisstudy,thepopulationof*F.oxysporum*on highly susceptible plants (*A. crassicarpa* and *F. moluccana*)wassignificantlyhigherthanotherplantsfor eachdiseasescore.Thispatterniscommonwherethe populationofpathogenisalsohigherwithdiseasescores (Scottetal.2014).deBorbaetal.(2017)reportedthat susceptiblelettucecultivarsshowedhigh *Fusarium* populationlevelandvulnerableblackbeangenotype showedapopulationlevelof15.4×10^5CFU^{g-1}.The secondpatternwasobservedon*L.leucocephala,*wherethe population of pathogen was also moderate with a moderate diseases score. A similar result was also occurred in garlic withdiseaseseverityof44%dueto *Fusarium*spp. infection, which showed a moderate number of pathogens on roots (Molinero-Ruiz et al. 2011).

Aspecialpatternoccurredon*A.pauciflorum*inwhich *F.oxysporum*causedamoderateinfection,butthe pathogenpopulationwaslow.Thismightbeduetothe plant defense mechanism. Scott et al. (2014)reported that resistant pepper plants also support pathogen development inroots,evenwithoutexternalsymptoms.Similarphenomenon wasreportedbyMuslimetal.(2003a)who notedthatsometomatoplantsareinfectedmoderately (score1–2)by *F.oxysporum*f.sp. *lycopersici*,butthe population was lower than other plants in same score.

The infection and total population on*Parkia speciosa* and*A.auriculiformis*werelower.Thisindicatedthatplants belonged to the resistant plant group. Fang et al. (2012) reported that when resistant strawberry plants were inoculated with*F. oxysporum* f. sp.*fragariae*, the cultivar formed a barrier with accumulated phenolic cells in the hypodermal layer that effectively limits the pathogen colonization and prevent the invasion of root vascular tissue. If the tissue penetration by hyphae was limited tothe epidermis, then the pathogens do notreach the vascular tissue. Van Den Berg et al. (2007) reported that banana clones tolerant to *F. oxysporum* f. sp.*cubense* correspond with this, with asignificantincreasein theinductionof cell wall-associated phenolic compounds. Jiménez-Fernándezet al. (2013) also reported that*Fusarium oxysporum* f. sp. *ciceris* race 0 remained in the intercellular space of root cortex and failed to reach xylem in resistant chickpea cultivars.

In this study, *A. crassicarpa* and *F. moluccana* were proventobeanalternativehostof*F.oxysporum*.Whereas *L.leucocephala, A.pauciflorum, P.speciosa*,and *A. auriculiformis*hadpotentialasalternativehosts.Many plantsofFabaceaefamilywereattackedbyformae specialis *F.oxysporum*, suchas *Vignaangularis*(*F. oxysporum* f.sp. *adzukicola*), *Cicerarietinum,Cicer*spp. (*F. oxysporum* f. sp. *ciceris*), *Acacia* spp. (*F. oxysporum* f. sp.*koae*),*Lens culinaris, L. esculenta* (*F. oxysporum*f. sp. *lentis*),*Medicago sativa* (*F. oxysporum*f. sp.*medicaginis*), *Phaseolusvulgaris, P.coccineus*(*F.oxysporum* f.sp. *phaseoli*),*Pisumsativum,Cicerarietinum*(*F.oxysporum*f. sp.*pisi*) (Edel-Hermann and Lecomte 2019). However, in this study,*F. oxysporum*isolatedfrom*A.mangium* has a wide host range from Fabaceae family; therefore, it is not classified as formae specialis.

In conclusion,*F. oxysporum*isolated from*A. mangium* causes infection in several types of forest and industrial plants.Since it has a wide host range, it isnot classified as part of the formae specialis group.

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