PERTANYAAN KLARIFIKASI KARYA ILMIAH NO. 5

5. Komentar untuk karya penelitian : 'Judul Artikel: A Rapid bioassay to evaluate efficacy of Hypovirulent Binucleate Rhizoctonia in reducing fusarium crown and root rot of tomato, Penulis: Dr. Ir. A. Muslim, M.Agr., Nama Jurnal: The Open Agriculture Journal, Volume Jurnal: 13, Nomor Jurnal: -, Tahun Terbit Jurnal: Maret 2019, Halaman: 27-33, ISSN: 1874-3315, Penerbit: BENTHAM Open': Karil terbit di The Open Agriculture Journal, Volume Jurnal: 13, Nomor Jurnal: -, Tahun Terbit Jurnal: Maret 2019, Halaman: 27-33, ISSN: 1874-3315, Penerbit: BENTHAM Open. Penerbit ini (BENTHAM Open) tergolong yang diragukan. Agar dapat diberikan Penilaian (tetapi tidak dapat menjadi karil syarat khusus), maka kepada pengusul wajib menyertakan/melampirkan buki-bukti proses korespondensinya (ejak submit sd published). Penilaian menunggu kelengkapan tsb.

JAWABAN KLARIFIKASI KARYA ILMIAH NO 5

Berikut kami lampirkan bukti-bukti korespondensi antara Author dengan Editorial Manager the Open Agriculture Journal yaitu sebagai Berikut:

1. Surat jawaban submision manuscript tgl. 7 Oktober 2018
2. Surat tanggapan untuk revisi manuscript dari Feer Reviewer: tgl. 31 Oktober 2018
3. Surat upload revisi manuscript dari Author: 14 November 2018
4. Surat sudah diterima revisi manuscript: 15 November 2018
5. Dokumen perbaikan pada manuscript dari Feer Reviewer
6. Dokumen Surat Revisi Perbaikan manuscript
7. Dokumen hasil revisi manuscript oleh Author
8. Surat permintaan Editor untuk memperbaiki grafik : 19 November 2018
9. Surat balasan perbaikan grafik : 26 November 2018
10. Surat Accepted paper: 6 December 2018.

BUKTI-BUKTI EMAIL DAN DOKUMEN KAMI LAMPIRKAN PADA HALAMAN BERIKUT.

1. JAWABAN SUBMISSION MANUSCRIPT

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<a_muslim@unsri.ac.id>

TOASJ Quick Track Notification | BMS-TOASJ-2018-43

1 message

The Open Agriculture Journal Sun, Oct 7, 2018 at 8:16 PM
<toasj@benthamopen.org>
Reply-To: The Open Agriculture Journal
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To: a_muslim@unsri.ac.id
Cc: toasj@benthamopen.org, wajeehaahmed@benthamopen.com, suwandi@fp.unsri.ac.id

Dear Dr. A Muslim,

Thank you very much for submitting your manuscript entitled "A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato " for consideration for publication in the "The Open Agriculture Journal".

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2. TANGGAPAN UNTUK REVISI MANUSCRIPT DARI FEER REVIEWER

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7 messages

The Open Agriculture Journal <admin@jms.eurekaselect.com>
Reply-To: The Open Agriculture Journal <toasj@benthamopen.org>
To: a_muslim@unsri.ac.id
Cc: wajeehaahmed@benthamopen.com

Wed, Oct 31, 2018 at 2:17 AM

Reference#: BMS-TOASJ-2018-43

Submission Title: A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato

Dear Dr. A Muslim,

Thanks for submitting the manuscript to "The Open Agriculture Journal". Your manuscript has been reviewed by experts in the field, and the consensus is that it needs significant revision keeping in consideration the comments given below. You are encouraged to address the comments of the reviewers and carefully revise the

manuscript, indicating the exact changes made in the manuscript.

1st Reviewer's Comments:

Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato" is quite interesting and provides much new information. The present research article is well written and seems to be a planned study. The present study was systematically evaluated efficacy of Hypovirulent Binucleate Rhizoctonia for Reducing Fusarium Crown and Root rot of Tomato. I believe that this research article shows considerable promise for the readers this journal globally. Research paper may be accepted after minor revision as below.

My suggestions for minor revision were:

1. Keywords should be minimum five in meaningful one or two words and arranged alphabetically.

2. Please follow the pattern of references as per Journal.

2nd Reviewer's Comments:

The observations are included in the manuscript (attachment).

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Authors who are native English speakers should ensure that their article has been revised for language, grammar, and style (where appropriate). This is in your interest as it will substantially reduce the time taken for publication of your article.

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Editorial Manager
E-mail: sahar@benthamopen.com
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Attachments:
Manuscipt A. Muslim et al The Open Agricultural Journal Rev ERF.docx |

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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Oct 31, 2018 at 9:58 AM
To: suwandi_unsri <suwandi_unsri@yahoo.com>, Suwandi fp <suwandi@fp.unsri.ac.id>

[Quoted text hidden]

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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Oct 31, 2018 at 10:02 AM
To: The Open Agriculture Journal <toasj@benthamopen.org>

Dear Prof.. Sahar Iftkehar

Thank you very much for your kindness to send us the review of our manuscript.
We are going to revise as soon as possible and send i back t you.

Thank you very much

Best Regard
Ahmad Musim

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To: Rahmat Pratama <rahmatpratamaunsri@gmail.com>

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Date: Wed, Oct 31, 2018, 02:17
Subject: TOASJ Manuscript Revision Required | BMS-TOASJ-2018-43
To: <a_muslim@unsri.ac.id>
Cc: <wajeehaahmed@benthamopen.com>

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3. UPLOAD REVISI MANUSCRIPT DARI AUTHOR

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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Nov 14, 2018 at 6:07 PM
To: The Open Agriculture Journal <toasj@benthamopen.org>

Dear Ms. Dr. Sahar Iftekhar
Editorial Manager

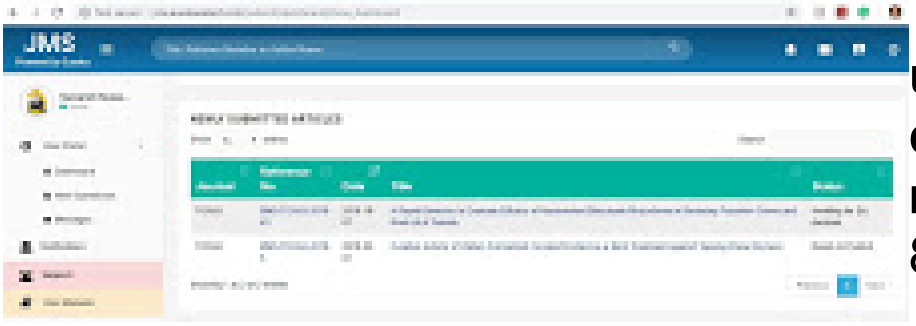
We have already send and upload our revised manuscript in title " A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root Rot of Tomato" through a system of The Open Agricultural Journal -TOASJ (enclosed).

I hope our manuscript could be processed for publishing in The Open Agricultural Journal -TOAS.

thank you very much for your kindness and excellent cooperation

Best Regard
A. Muslim
Sriwijaya University

[Quoted text hidden]



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4. TANGGAPAN REVISI MANUSCRIPT SUDAH DITERIMA

The Open Agriculture Journal <toasj@benthamopen.org> Thu, Nov 15, 2018 at 6:20 PM
To: "a. muslim unsri" <a_muslim@unsri.ac.id>
Cc: qasit@benthamopen.com, Bnetham Open - Sahar Iftakhar <sahar@benthamopen.com>

Dear Dr. A. Muslim,

Many thanks for your email. We have safely received your revised manuscript entitled “**A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate**

Rhizoctonia in Reducing Fusarium Crown and Root Rot of Tomato" and sent for re-reviewing.

You will be informed on the final editorial decision.

Regards,

Wajeeha Ahmed

Assistant Manager(Publication)

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a. muslim unsri <a_muslim@unsri.ac.id> Fri, Nov 16, 2018 at 7:13 AM
To: The Open Agriculture Journal <toasj@benthamopen.org>

Dear Dr. Wajeeha Ahmed
Asistant Manager (Puublication)

Thank you very much for your excellent respond.

We hope our paper entitled " A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate *Rhizoctonia* in Reducing Fusarium Crown and Root rot of Tomat" could be published in your journal of TOASJ soon.

Thank you very much for your excellent cooperation.

Best ragard
Ahmad Muslim
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5. DOKUMEN UNTUK PERBAIKAN MANUSCRIPT DARI FEER REVIEWER

A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate *Rhizoctonia* in Reducing Fusarium Crown and Root rot of Tomato

A. Muslim[1], Mitsuro Hyakumachi[2], Koji Kageyama[3], Suwandi Suwandi[1], and Rahmat Pratama[1]

[1)] Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Jl. Raya Palembang-Prabumulih, Km. 32, Inderalaya, Ogan Ilir 30662, Indonesia
[2)] Laboratory of Plant Disease Science, Faculty of Agriculture, Gifu University, Yanagido 1-1,501-1193 Gifu, Japan.
[3)] River Basin Research Center, Gifu University, Gifu 501-1193, Japan.

Abstract:

Background:
Fusarium oxysporum f. sp. *radicis-lycopersici* (FORL) caused Fusarium crown and root rot of tomato (FCRR), it's a serious constraint on tomato production and contributing to yield losses.

Aims/Method:
Using a rapid bioassay, hypovirulent binucleate *Rhizoctonia* (HBNR) were tested for their ability to reduce fusarium crown and root rot (FCRR) of tomato, caused by *Fusarium oxysporum* f.sp. *radicis lycopersici* (FORL). Roots of tomato seedlings growing on 2 % water agar in plastic boxes were inoculated with living or dead mycelial disks of HBNR. After 24 h, the pathogen was applied at 0, 3, 6, and 9 cm away from the position of the HBNR.

Result
When living HBNR was used, the treatments provided significant protection to tomato seedlings from FCRR infection at all distances tested. Tomato plants pre-inoculated with living HBNR at different times (12 h and 24 h before inoculation with the pathogen) and challenged with FORL showed significant reduction of FCRR lesion development. Significant reduction was still observed even when HBNR was inoculated simultaneously with or 12 h after inoculation of pathogen. Seedlings treated with dead HBNR and culture filtrates also showed significantly reduced FCRR lesion development. When living HBNR were enveloped by polycarbonate membrane filter, significant reduction of FCRR lesion development was still observed. In all experiments, reduction of FCRR lesion development in seedlings treated with HBNR tended to decrease with longer distance from the inoculation point of FORL and HBNR. We developed a simple, rapid, and miniaturized bioassay for evaluating the efficacy of HBNR against FORL. The bioassays require only 12 - 18 days, which is at least 12 days less than the soil system employed by previous researchers.

Keywords: Hypovirulent Binucleate *Rhizoctonia*, *Fusarium oxysporum* f.sp. *radicis-lycopersici*, Tomato, Rapid Bioassay

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* Address correspondence to this author at the Department of Plant Protection, Faculty of Agriculture, Sriwijaya University, Jl. Palembang-Prabumulih Km.32 Indralaya, Palembang 30662, Indonesia; Tel: +6281367769589; Fax: 62-711-580644; E-mails: a_muslim@unsri.ac.id, limpal2003@yahoo.com

1. INTRODUCTION

Fusarium crown and root rot of tomato (FCRR), caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), is a serious constraint on tomato production that limits the yield of glasshouse- and field-grown tomato crops [1]. The disease was first detected in Japan in 1974 [2]. Yield losses caused by FCRR were 33 % and 44 % in Hokkaido and Kochi Prefectures, respectively [3]; [4].

Recent research on the management of Fusarium wilt and FCRR has focused on diverse strategies, either individually or in combination. These strategies include host resistance and chemical, biological, and physical control [5]. [6] demontrated that grafting tomato hybrid plants onto "Natalia" rootstock significantly enhanced the tolerance of plants to FORL, even though proteomic analysis showed a higher representation of proteins associated with pathogen infection. A combination of a plant-growth-promoting strain of *Fusarium equiseti* with biodegradable pots was also an effective control of FCRR [7].

Several studies have demonstrated that *Pseudomonas* sp. strain FC-24B, *P. putida* FC-8B [8] and *P. chlororaphis* [9] effectively reduced *Fusarium oxysporum* f. sp. *radicis-lycopersici*. In a study using four rhizospheric bacteria (*Bacillus*, *Lysinibacillus*, *Enterobacter*, and *Serratia*) and one root-associated endophytic (RAE) associated with *Alcaligenes faecalis* subsp. caused a statistically significant decrease in plant infection by FORL through antibiosis mechanisms [10]. [11] reported that *Pseudomonas fluorescens* WCS365 and *P. chlororaphis* PCL1391 effectively controlled FCRR through induced systemic resistance.

Hypovirulent binucleate *Rhizoctonia* (HBNR) were investigated as effective biocontrol agents for a number of important diseases caused by *Rhizoctonia solani* [12] and *Phytium* [13]. Our previus research showed that HBNR effectively controls Fusarium wilt of tomato [14], Fusarium wilt of spinach [15], and Fusarium crown and root rot of tomato [16]. These studies indicated that one of the mechanisms of biocontrol of fusarium diseases with HBNR isolates might be induced resistance. Investigations of HBNR as an agent of induced systemic resistance (ISR) in beans, against the root rot pathogen *Rhizoctonia solani* or the anthracnose pathogen *C. lindermuthianum*, have also been reported [17]. HBNR also effectively protected cotton seedlings against rhizoctonia damping-off and alternaria leaf spot with mechanism of induced systemic resistance (ISR) [18].

A major limiting factor in the development of biological control strategies for different plant diseases is the formulation of efficient procedures for rapidly screening large numbers of organisms for biological control activity. While field screening should theoretically provide the best detection of efficient biocontrol strains, limitations of space, labor, cost, and optimal environmental conditions preclude the use of this type of screening strategy. Laboratory assays based on the *in vitro* inhibition of pathogens or production of particular metabolites by biological control agents offer a rapid and relatively inexpensive means of screening organisms but may not be good indicators of biocontrol potential. Unsurprisingly, biocontrol strains selected *in vitro* on the basis of phenotypes with unknown links to biological control activity in plant systems do not always perform as expected under greenhouse or field conditions [19];[20]. The present study was undertaken to: (1) develop a rapid and miniaturized laboratory bioassay for screening the efficacy of HBNR in reducing FCRR in the tomato; (2) investigate the efficacy of various inoculum forms of HBNR in controlling FCRR using a water agar system.

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2. MATERIALS AND METHODS

Organisms: Four isolates of HBNR were used as biocontrol agents: L1 (AG-Ba), W1, W7 (AG-A), and HBNR Rhv7 (unknown anastomosis group). *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) isolate RJNI, obtained from a tomato infested with fusarium crown and root rot (FCRR), was used as the inoculum of the pathogen.

Plant: Tomato cv. "House Momotaro", a popular cultivar that is susceptible to FCRR, was used throughout the experiments.

Inoculum preparation: (1) The pathogen, FORL, was grown on potato dextrose agar (PDA) for 7 days in the dark at 25 ^{o}C. Spores were scraped from the cultures with a sterile glass bar, and a spore suspension was prepared in sterile water and filtered through eight layers of sterile gauze. (2) HBNR isolates were prepared as inoculum forms in potato dextrose agar (PDA) plugs (living and dead mycelial disks). The isolates were grown on PDA for 3-7 days in the dark at 25 ^{o}C. The dead mycelial disk was prepared by killing the 7-day-old culture with chloroform and then drying it for 60 min on a clean bench. To make culture filtrate (CF), two mycelial disks of each HBNR isolate, obtained from the growing margin of a colony on PDA, were transferred to a 200-ml flask containing 50 ml of potato dextrose broth (pH 6.5). The isolates were cultured without shaking for 10 days in dark. The crude culture filtrate was separated from mycelia and filtered three times through three layers (each time) of Whatman no. 2 filter paper. The CF was then filter sterilized (0.45-µm Millipore filters, Millipore Products Division, Bedford, USA).

2.1. Laboratory assay of biological control of Fusarium crown and root rot of tomato

The efficacy of HBNR in suppressing the development of FCRR in the tomato was tested in laboratory experiments using a water agar (WA) system method (Fig. 1). Tomato seeds were surface-sterilized in 70 % ethyl alcohol for 1 min followed by soaking in 1 % sodium hypochlorite with 3 drops of Tween 20 (polyoxyethylene sorbitan monolaureate; Nacalai Tesque, Inc., Kyoto, Japan) for 20 min. The seeds were then rinsed three times with sterilized distilled water (SDW). The seeds were pre-germinated on 2 layers of Whatman No. 1 filter paper for 3 days in the dark at 25 ^{o}C. Five seedlings were transferred to a sterilized plastic box (196 × 104.5 × 28 mm) containing water agar (WA) and allowed to grow for 6 days at about 20 in a cleanroom. A living HBNR mycelial disk (3-mm diameter, taken from the advancing margin of a three-day-old culture), a dead mycelial disk (7-mm diameter), and CF (70 µl) were used to inoculate the basal hypocotyls of the seedlings, which were again incubated for 24 h. To prevent spread and maintain a uniform distribution of CF on basal hypocotyls or roots, drops of CF were placed on an 8-mm diameter paper disc with 1.5-mm thickness (Advantec, Toyo Roshi Kaisha, Ltd. Japan). To avoid direct contact between HBNR and FORL, the mycelial disk of HBNR was enveloped by a polycarbonate membrane filter (0.2-µm mesh). An additional experiment using a living mycelial disk (3-mm diameter) without an enveloping membrane was also done. In this experiment, the inoculation period of the HBNR varied from 0 h to 12 h after inoculation with the pathogen and 0 h to 24 h prior to inoculation with the pathogen. As a control, seedlings were inoculated with HBNR-free PDA or SDW. Then, 5 µl of pathogen suspension (5 × 10^5 spores/ml) were inoculated at positions 0, 3, 6, and 9 cm away from the position of the HBNR inoculum. A 5-mm diameter disk of lens

Commented [E7]: Explain the differences of each HBNR isolate.

Commented [E8]: According to the method used, it is not a simple and rapid bioassay, nor is it cheap.

Commented [E9]: Why try the dead mycelium? Explain.

paper was placed on each drop to prevent runoff and to maintain a uniform distribution of spores on the root surface. The treatments were prepared in four replicates. Treated and control seedlings were maintained at about 20 ^{o}C for another 2-10 days. Disease severity was determined by measuring lesion development at the pathogen inoculation point. Percent reduction of lesion development was used to measure the efficacy of HBNR against the pathogen, by employing the formula [(A-B)/A] x 100, in which A represents the lesion length observed on the root due to inoculation of pathogen alone and B is the lesion length observed on the root due to co-inoculation of HBNR and the pathogen.

Commented [E10]: How was the lesion size measured? Did they only use a ruler, or was a vernier used? It is very important, because the work is based on the results of the measurement of the lesion.

2.2 Data analysis

The experiments were carried out in completely randomized design. Treatment means obtained for lesion development of FCRR were compared using Fisher's least significant difference (LSD) test with critical values of $P = 0.05$ and $P = 0.01$.

3. RESULTS

3.1 Biological control of FCRR of tomato with HBNR

In a WA system, tomato seedlings treated with living mycelia, dead mycelia, and CF of HBNR isolates significantly reduced lesion development of FCRR ($P = 0.05$).

When living mycelia were used as treatment, seedlings treated with HBNR isolates had significantly less FCRR lesion development after 4 - 10 days of pathogen inoculation ($P = 0.01$; Fig. 2). The percentage of reduction tend to decrease with the longer distance between HBNR and FORL. At a distance of 0 cm between HBNR and FORL, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was almost completely ranged from 81 – 96 %. At a distance of 3 cm, application of all HBNR still higly reduced lesion development by 72 – 91 %. At a distance of 6 cm and 9 cm, the reduction of lesion development by all HBNR isolates slightly decreased by 25 – 84 % and 35 – 75 %, respectively (Fig. 2).

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Tomato seedlings treated with dead mycelia of all HBNR isolates except L2 also showed significant reduction of FCRR lesion development 5 days after inoculation with the pathogen ($P = 0.05$; Fig. 3). At a distance of 0 cm, lesion development reduction was 19 %, 62 %, 41 %, and 30 % for HBNR L2, W1, W7, and Rhv7, respectively (Fig. 3A). At a distance of 3 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 18 %, 52 %, 32 %, and 34 %, respectively (Fig. 3B). At a distance of 6 cm, lesion development reduction was 21 %, 38 %, 42 %, and 32 % for HBNR L2, W1, W7, and Rhv7, respectively (Fig. 3C).

The application of CF of HBNR isolates also resulted in significant reduction in FCRR lesion development 2-8 days after pathogen inoculation ($P = 0.05$; Fig. 4). At a distance of 0 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 35 – 100 %, 36 – 100 %, 37 - 100%, and 36 - 100%, respectively (Fig. 4A). At a distance of 3 cm, treatment with HBNR L2, W1, W7, and Rhv7 reduced lesion development by 30 – 87 %, 31 – 100 %, 22 – 100 %, and 27 - 100%, respectively (Fig. 4B). At a distance of 6 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 20 – 70 %, 33 – 100 %, 26 – 100 %, and 27 – 100 %, respectively (Fig. 4C).

Commented [E12]: Why do they mention that there was a 100% reduction of lesion? If, on day 2, the disease just begins to develop, it is not controlled.

Commented [E13]: Same.

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We attempted to prevent direct contact between HBNR and FORL by enveloping the living mycelia in a polycarbonate membrane filter (0.2-µm mesh), but

the mycelia still penetrated the membrane, so that direct contact between HBNR and FORL was observed. In this experiment, significant reduction in FCRR lesion development was still observed 4 - 6 days after pathogen inoculation at a distance of 0 - 3 cm (P = 0.05; Fig. 5A, 5B). However, at a distance of 6 cm, significant reduction was only observed at 4 days after pathogen inoculation (Fig. 5C). The reduction of lesion development by HBNR W1 was 34 – 61 %, 45 – 57 %, and 2 – 36 % at distances of 0, 3, and 6 cm, respectively.

In another experiment, pre-inoculation at 12 h and 24 h with living mycelia of HBNR W1 or Rhv7 on the seedlings, and challenge-inoculation with FORL at 3 cm and 6 cm away from HBNR, also resulted in significant reduction in lesion development compared to the control, after 8 days of pathogen inoculation (Table 1). At 12 h pre-inoculation of HBNR, at a distance of 3 cm, treatment with HBNR W1 and Rhv7 reduced FCRR lesion development by 90 % and 91 %, respectively. At a distance of 6 cm, the reduction by HBNR W1 and Rhv7 was 71 % and 71 %, respectively. The reduction slightly increased with the longer pre-inoculation period of 24 h. At a distance of 3 cm, the reduction by HBNR W1 and Rhv7 was 93 % and 90 %, respectively. At a distance of 6 cm, the reduction by HBNR W1 and Rhv7 was 82 % and 74 %, respectively. HBNR isolates also significantly reduced lesion development of FCRR (P = 0.01) when both isolates were applied simultaneously (0 h) and even when HBNR was applied 12 h after pathogen inoculation. At 0 h, or simultaneous inoculation, at a distance of 3 cm, the reduction of lesion development by HBNR W1 and Rhv7 was 89 % and 90 %, respectively. At a distance of 6 cm, the reduction was 71 % and 64 % for HBNR W1 and Rhv7, respectively. At 12 h after pathogen inoculation, at a distance of 3 cm, the reduction was 89 % and 81 % for HBNR W1 and Rhv7, respectively. At a distance of 6 cm, the reduction by HBNR W1 and Rhv7 was 66 % and 59 %, respectively.

Commented [E15]: This treatment is not explained in the methods, it seems that they included it after the initial experiment; therefore, it is not explained or justified because they did it.

Commented [E16]: For both.

4. DISCUSSION

In the experiment using the WA system method, inoculation of the living HBNR mycelia on the base hypocotyls, and the pathogen on a different site 0, 3, 6, and 9 cm away from HBNR, showed that all HBNR isolates tested significantly reduced FCRR lesion development. Maximum protection occurred when the pathogen was inoculated at the position of 0 and 3 cm away. However, protection decreased a little bit at a distance of 6 and 9 cm. In this system, although the pathogen was directly introduced to the root surface, high lesion reduction was still provided by HBNR. Pre-inoculation of living mycelia of HBNR at a different time, and challenged with FORL, resulted in significant reduction in FCRR lesion development, even when HBNR was inoculated simultaneously (0 h) or 12 h after inoculation of pathogen. These results support those obtained by [21]: *Rhizoctonia* damping-off in bedding plants was still reduced when binucleate *Rhizoctonia* was applied together with *R. solani* AG-4 and AG-8. [22] also reported that application of *Trichoderma harzianum* Th-3013 was still able to control purple blotch disease even when performed 48 h after pathogen inoculation. However, a contrary result was reported by [23] tomato seedlings treated with non-pathogenic *Fusarium* 7 or 14 days before inoculation of the pathogen showed the greatest effect. However, the protective effect almost disappeared when both were applied simultaneously. The different results achieved by different researchers might be caused by a difference in the mechanisms of disease suppression involved in the varying system.

Commented [E17]: They are results, not discussion.

Commented [E18]: Reference 21 and 23 very old.

Commented [E19]: There is no discussion, they only make a comparison with other studies.

Tomato seedlings treated with CF and dead mycelia of HBNR effectively reduced FCRR lesion development. The *in vitro* interaction experiments using living

or dead mycelia and CF reveal that they did not produce any zone of inhibition, suggesting that they were not antagonistic and ruling out the possible involvement of toxins or antifungal compounds in disease suppression. Since CF and dead mycelia of HBNR application sites and pathogen application sites were spatially separated by a distance of 3-6 cm, and there was no contact between HBNR isolates and the pathogen until day 5 at 3 cm and day 8 at 6 cm, we observed that average mycelial growth of the pathogen was 0.54 cm/day. Induced resistance in tomato plants by HBNR may be one of the mechanisms of biological control against FCRR in this study. These results confirm those of [24] and [25], who reported that HBNR did not inhibit or parasitize *R. solani*. Many reports demonstrated that mycelia or CF of fungi were effective in inducing resistance against various diseases [26];[27];[28]. [29] demonstrated that tomato plants treated with Oligandrin, the elicitin-like protein produced by the mycoparasite *Pythium oligandrum*, showed significant induction of systemic resistance against FORL. The most striking features of the resistance mechanism involved restriction of fungal growth to the outer root tissues, decrease in pathogen viability, and formation of aggregated deposits, which often accumulated at the surface of invading hyphae. In addition, [30] reported that cucumber seedlings treated with pectinases extracted from fermentation products of *Penicillium oxalicum* BZH-2002 induced resistance against scab caused by *Cladosporium cucumerinum*.

Various bioassays for screening biocontrol agents use soil systems [14];[16];[31], and other bioassays for induced resistance in tomato plants have been reported, such as split root, benomyl, cutting, and layering [32]. However, these systems, like most other bioassays used for screening of biocontrol agents, often require more than one month to complete. Such long-term bioassays are difficult to use in large screening trials. In contrast, the bioassay used in this study offers the advantage of a short assay period (12 - 18 days) and requires only a small amount of space in cleanroom to test many different strains or isolates. Another advantage of this assay was its simplicity and the need for only small amounts of biocontrol agent and pathogen inoculum. By screening strains initially on plants, as opposed to pathogen-inhibition assays in Petri dishes, we hope to minimize the erroneous selection of strains on the basis of biological control traits that would not be expressed in more complex ecosystems.

The results presented in this study establish that this rapid bioassay can be might also effectively to screen large numbers of microorganisms as biocontrol agents and for induce resistance activity. We expect that the bioassay used in this study could be also useful as a rapid assay in pathogenicity testing of FCRR.

CONCLUSIONS

In this experiment using the Water Agar system method, tomato seedlings treated with living mycelia, dead mycelia, and CF of HBNR isolates and *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) were inoculated at positions 0, 3, 6, and 9 cm away from the position of the HBNR resulted in significantly reduced lesion development of FCRR. The reduction of lesion development of FCRR decreased with the longer distance between HBNR and FORL. Pre-inoculation of living mycelia of HBNR at a different time, and challenged with FORL, resulted in significant reduction in FCRR lesion development, even when HBNR was inoculated simultaneously (0 h) or 12 h after inoculation of pathogen. Laboratory assay developed in this study markedly shortened the time needed for evaluating the ability of HBNR to control FCRR. This assay requre only 12 - 18 days from seedling appearance to rating for disease severity, which is at least 12 days less than the soil

Commented [E20]: It is necessary to check it.

Commented [E21]: Very old references. 31 years later, there is much more information regarding the subject.

Commented [E22]: This paragraph should be passed to the introduction, to justify the implementation of the assay they propose.

Commented [E23]: Induce resistance activity or induced systemic resistance (ISR)?

Commented [E24]: In this paragraph, results are presented, they are not conclusions.

system employed by previous researchers. This method was also simple and least demanding of space and growth facilities.

Commented [E25]: It is not concluded about the objective (2): L1 (AG-Ba), W1, W7 (AG-A), and HBNR Rhv7

ACKNOWLEDGMENTS

We thank the Ministry of Education, Science, Sports, and Culture (Monbukagakusho) Japan, for financial assistance.

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Fig.(1). Diagram of laboratory assay of hypovirulent binucleate *Rhizoctonia* (HBNR) to suppress the disease development of Fusarium crown and root rot (FCRR) of tomato and to induce resistance against the disease, using the water agar method. (**A**) Inoculation point of HBNR consisting of a living mycelial disk (3-mm diameter), a dead mycelial disk (7-mm diameter), and CF (70 µl). In order to avoid direct contact between HBNR and FORL, the mycelial disk of living cells was enveloped by a polycarbonate membrane filter (0.2-µm mesh); (**B**) Inoculation point of *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) with spore suspension (5 µl of pathogen suspension at 5×10^5 spores/ml) at 0, 3, 6, and 9 cm away from the position of HBNR inoculum (separate experiment for each position); (**C**) Tomato root.

Commented [E26]: The scale of the figure is inadequate, the size of the root in relation to the leaf area does not correspond. What is the support of the seedling?

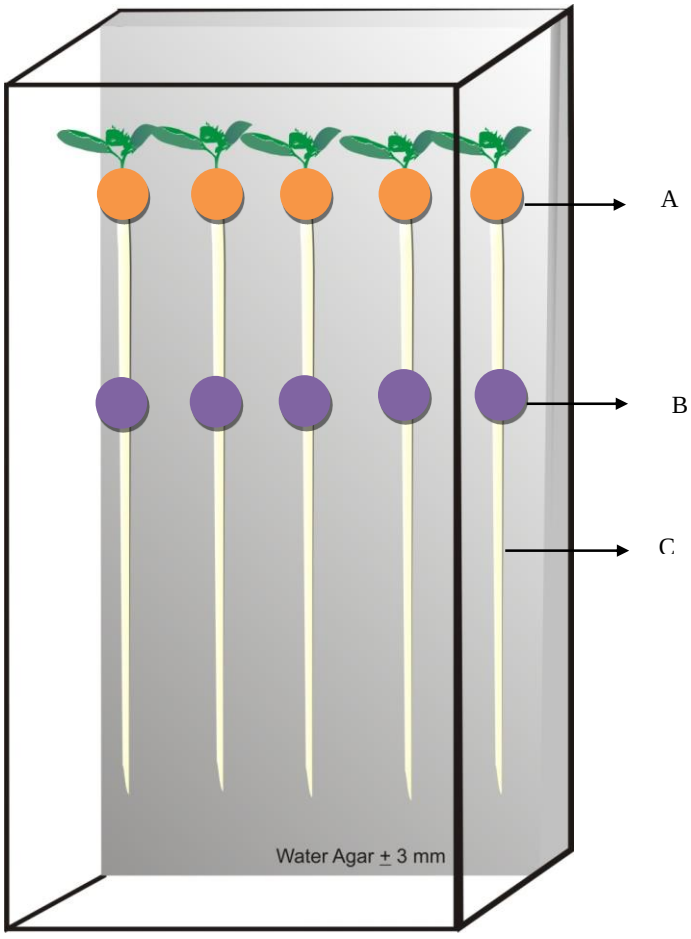

Fig.(2). Effect of living mycelia of HBNR isolates on lesion development of Fusarium crown and root rot of tomato, after being challenge-inoculated with FORL at 0 cm (**A**), 3 cm (**B**), 6 cm (**C**), and 9 cm (**D**) away from the position of HBNR inoculum. Data are means of 4 replications with 5 seedlings per replication.

Commented [E27]: In Figure 3 the measurement was made on day 5, in this figure measurements are made from day 2 to 10, why the difference? It is the same system.

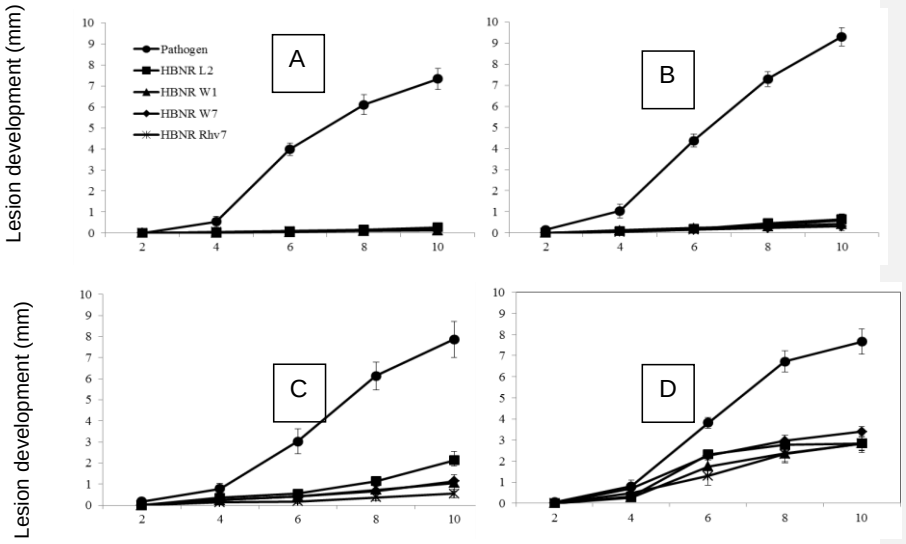

Fig.(3). Effect of dead mycelia of HBNR isolates on lesion development of Fusarium crown and root rot of tomato, after being challenge-inoculated with FORL at 0 cm (**A**), 3 cm (**B**), and 6 cm (**C**) away from the position of HBNR inoculum. Data are means of 4 replications with 5 seedlings per replication. Data were recorded 5 days after pathogen inoculation. Bars labeled with the same letter are not significantly different according to Fisher's least significant different test ($P > 0.05$).

Commented [E28]: Why is this figure recorded on day 5?

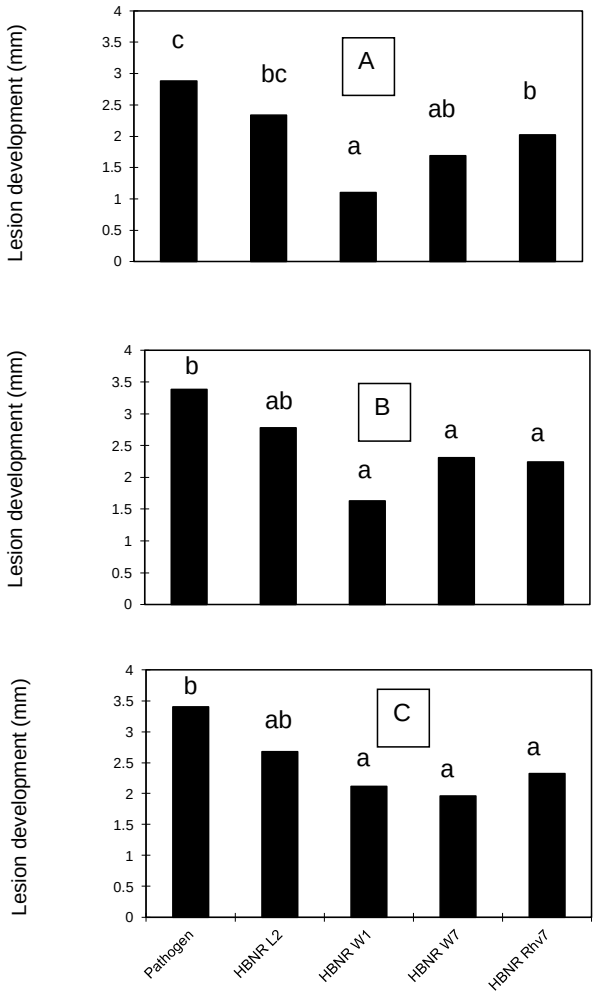

Fig.(4). Effect of culture filtrates of HBNR isolates on lesion development of Fusarium crown and root rot of tomato after challenge-inoculation with FORL at 0 cm (**A**), 3 cm (**B**), and 6 cm (**C**) away from the position of HBNR inoculum. Data are means of 4 replications with 5 seedlings per replication. Bars represent standard error of the mean.

Commented [E29]: In figure 4 the measurements were made from day 2 to 8, in figure 2 they were made from 2 to 10. If it is the same system, why the differences in the taking of measurements?

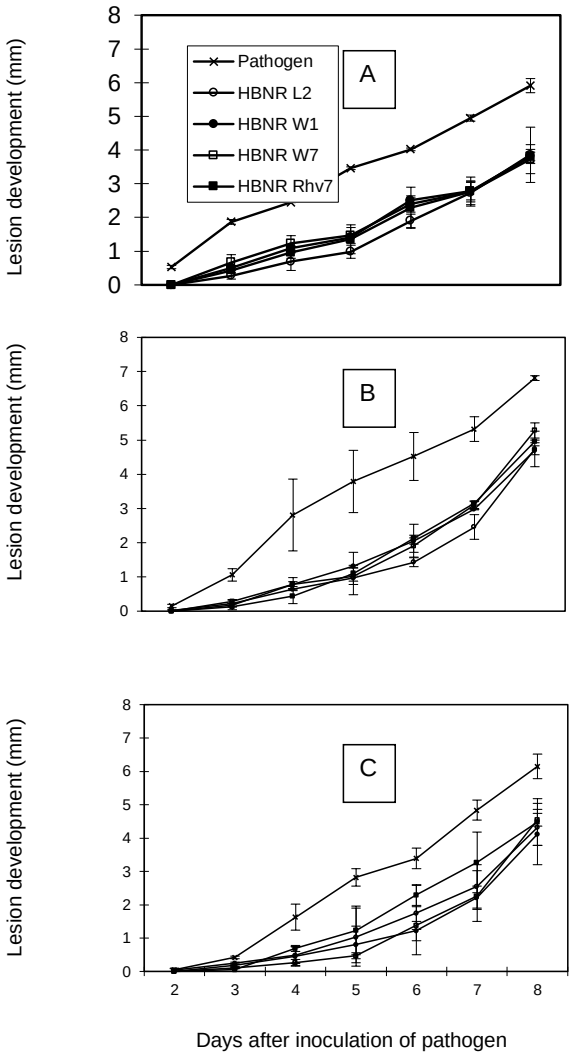

Fig (5). Effect of living mycelia of HBNR isolates covered with polycarbonate membrane filter (0.2-µm mesh) on lesion development of Fusarium crown and root rot of tomato after challenge-inoculation with FORL at 0 cm (**A**), 3 cm (**B**), and 6 cm (**C**) away from the position of HBNR inoculum. Data are means of 4 replications with 5 seedlings per replication. Bars labeled with the same letter are not significantly different according to Fisher's least significant different test ($P > 0.05$).

Commented [E30]: In Figure 5, why are only the results of HBNR W1 reported?

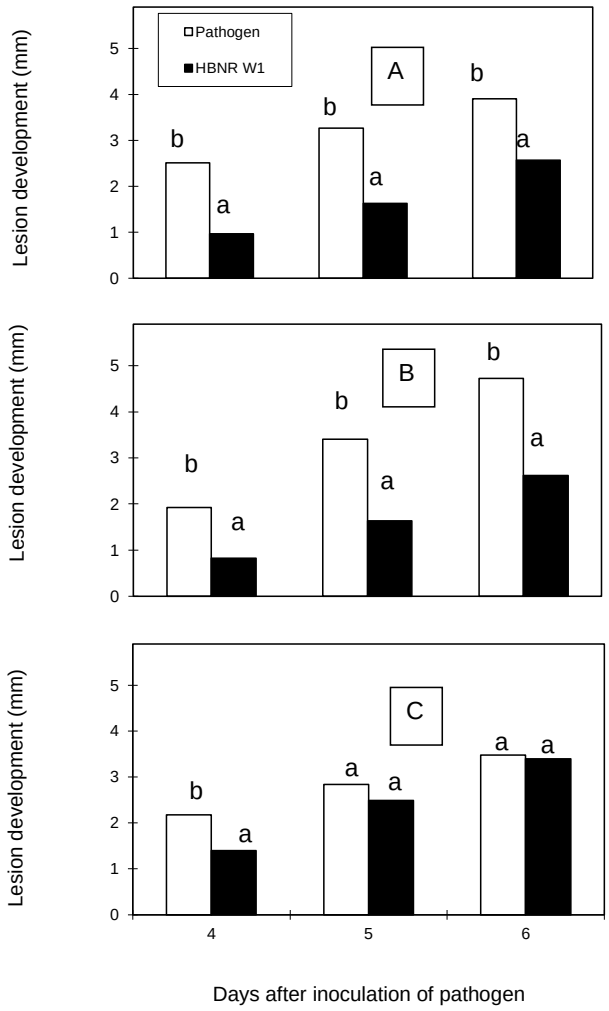

Table 1. Effect of hypovirulent binucleate *Rhizoctonia* (HBNR) with various pre-incubation times on the reduction of lesion development of Fusarium crown and root rot (FCRR) of tomato caused by *Fusarium oxysporum* f. sp. *radicis lycopersici* (FORL) in water agar [a]

Treatments	Lesion development (cm)[b]							
	3 cm[c]				6 cm			
	-12[d]	0	12	24	-12	0	12	24
Pathogen	7.2 b[e]	7.0 b	6.7 b	7.0 b	6.4 b	6.1 b	5.6 b	6.1 b
HBNR W1	0.8 a	0.8 a	0.7 a	0.5 a	2.2 a	1.8 a	1.6 a	1.1 a
HBNR Rhv7	1.4 a	0.7 a	0.6 a	0.7 a	2.6 a	2.2 a	1.6 a	1.6 a

[a] Eight-day-old tomato seedlings were grown in 2 % water agar treated with HBNR and challenge-inoculated with FORL.
[b] Lesion development was recorded 8 days after inoculation with FORL.
[c] Inoculation points of FORL were 3 cm and 6 cm away from HBNR position.
[d] Pre-incubation of HBNR on neck root: 12 h after inoculation of pathogen (-12); simultaneous inoculation of HBNR and pathogen (0 h); 12 h before inoculation of pathogen (12); 24 h before inoculation of pathogen (24).
[e] Mean of four replications with five seedlings per replication. Values followed by the same letter do not differ significantly ($P > 0.01$) according to Fisher's least significant difference test.

Commented [E31]: Why is reported in table 1 the size of the lesion in cm, and in the previous graphs in mm?. There was more lesion in this assay? Why are only results from W1 and Rhv7 reported?

Commented [E32]: The information must be included in the methods section.

6. SURAT TANGGAPAN REVISI MANUSCRIPT DARI AUTHOR

Dear Ms. Sahar Iftekhar
Editorial Manager, The Open Agriculture Journal

Thank you very much for your generous suggestions. We have revised the manuscript according to your suggestion and the two reviewer.

1. **Keywords: Hypovirulent Binucleate** *Rhizoctonia, Fusarium oxysporum f.sp. radicis-lycopersici,* **Tomato,** *Rapid Bioassay*
   Preferably, the keywords must be different from the words included in the title.

   Answer
   We agree, we propose the keywords are: Evaluation of biological control agents, *Fusarium oxysporum f.sp. radicis-lycopersici,*

2. glasshouse change to Greenhouse

   Answer
   We agree to change glasshouse to be Greenhouse
3. [3]; [4]. Include more recent studies to justify the losses in tomato production.

   Answer
   We include the recent study: Yield losses due to FCRR in greenhouse and field tomato production range from 15 to 65% (Ozbay and Newman 2004).

   Ozbay, N and Newman, S.E. 2004. Fusarium Crown and Root Rot of Tomato and Control Methods. Plant Pathology Journal 3 (1): 9-18.

4. Information not necessary “Several studies have demonstrated that *Pseudomonas* sp. strain FC-24B, *P. putida* FC-8B [8] and *P. chlororaphis* [9] effectively reduced *Fusarium oxysporum* f. sp. *radicis-lycopersici*. In a study using four rhizospheric bacteria (*Bacillus, Lysinibacillus*, *Enterobacter*, and *Serratia*) and one root-associated endophytic (RAE) associated with *Alcaligenes faecalis* subsp. caused a statistically significant decrease in plant infection by FORL through antibiosis mechanisms [10]. [11] reported that *Pseudomonas fluorescens* WCS365 and *P. chlororaphis* PCL1391 effectively controlled FCRR through induced systemic resistance”.

   Answer
   We agree to remove this paragraph

5. According to the background, it is already proven that HBNR controls FCRR [(1) develop a rapid and miniaturized laboratory bioassay for screening the efficacy of HBNR in reducing FCRR in the tomato].

   Answer
   We agree that HBNR have been proven effectively control FCRR according to the background. Since we develop a rapid method to evaluate HBNR against FCRR in laboratory

assay in particular for selecting a large number of HBNR, therefore it is still necessary to mention [develop a rapid and miniaturized laboratory bioassay for screening the efficacy of HBNR in reducing FCRR in the tomato] as our objective.

6. Write what are the inoculum forms used in the objection 2 "(2) investigate the efficacy of [various] inoculum forms of HBNR in controlling FCRR using a water agar system".

Answer
We agree the inoculum forms used to be included in our manuscript. We rewrite the objective no 2 to be (2) investigate the efficacy of various inoculum forms (living and dead mycelial disks) of HBNR in controlling FCRR using a water agar system.

7. Explain the differences of each HBNR isolate in the MATERIALS AND METHODS Organisms: "Four isolates of HBNR were used as biocontrol agents: L2 (AG-Ba), W1, W7 (AG-A), and HBNR Rhv7 (unknown anastomosis group)".

Answer:
We have already explained the differences of isolate in the parentheses such as, L2 (AG-Ba) means that L2 belong to the anastomosis group Ba.

8. According to the method used, it is not a simple and rapid bioassay, nor is it cheap. "Laboratory assay of biological control of Fusarium crown and root rot of tomato"

Answer
When we compare to the ordinary evaluation of HBNR against plant disease in greenhouse which is need seedling preparation (21 days) in small pot then were transferred to bigger plastic pot containing pathogen-infested soil medium for recording of disease severity for about 70 days. In this study we just need cheap materials (plastic box and water agar) and we just need only nine days for seedling preparation and ten days for diseases recording.

9. A living HBNR mycelial disk (3-mm diameter, taken from the advancing margin of a three-day-old culture), a dead mycelial disk (7-mm diameter) "Why try the dead mycelium? Explain"

Answer:
Beside living cell of antagonist use as biocontrol agent, dead cell such as dead mycelium also can be used as effective biocontrol agent as reported by:
Zhang, H.J., Dong, H.Z., and Li, W.J. 2011. Dead mycelium of *Penicillium chrysogenum* protects transplanted cotton plants against fungal wilts in a saline field. Spanish Journal of Agricultural Research 9(3): 873-881.

10. Percent reduction of lesion development was used to measure the efficacy of HBNR against the pathogen, by employing the formula □(A-B)/A□ x 100, in which A represents the lesion length observed on the root due to inoculation of pathogen alone and B is the lesion length observed on the root due to co-inoculation of HBNR and the

pathogen. “How was the lesion size measured? Did they only use a ruler, or was a vernier used? It is very important, because the work is based on the results of the measurement of the lesion”.

Answer:
Lesion length was measured using a Vernier caliper

11. At a distance of 0 cm between HBNR and FORL, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was almost completely ranged from 81 – 96 %. “In the methods it appears as L1”.

Answer:
We apologize for mistyping L1 in the Methods. The correct one is L2.

12. At a distance of 0 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 35 – 100 %, 36 – 100 %, 37 - 100%, and 36 - 100%, respectively (Fig. 4A). At a distance of 3 cm, treatment with HBNR L2, W1, W7, and Rhv7 reduced lesion development by 30 – 87 %, 31 – 100 %, 22 – 100 %, and 27 - 100%, respectively (Fig. 4B). At a distance of 6 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 20 – 70 %, 33 – 100 %, 26 – 100 %, and 27 – 100 %, respectively (Fig. 4C). “Why do they mention that there was a 100% reduction of lesion? If, on day 2, the disease just begins to develop, it is not controlled.

Answer
Thank you very much for your excellent suggestion. We have rewrite with the percentage lesion reduction from day 4^{th}.

At a distance of 0 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 35 – 85 %, 36 – 73 %, 37 - 100%, and 36 - 64%, respectively (Fig. 4A). At a distance of 3 cm, treatment with HBNR L2, W1, W7, and Rhv7 reduced lesion development by 30 – 79 %, 31 – 83 %, 23 – 74 %, and 27 - 88%, respectively (Fig. 4B). At a distance of 6 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 30 – 70 %, 33 – 72 %, 26 – 84 %, and 27 – 86 %, respectively (Fig. 4C). “Why do they mention that there was a 100% reduction of lesion? If, on day 2, the disease just begins to develop, it is not controlled.

13. In another experiment, pre-inoculation at 12 h and 24 h with living mycelia of HBNR W1 or Rhv7 on the seedlings, and challenge-inoculation with FORL at 3 cm and 6 cm away from HBNR, also resulted in significant reduction in lesion development compared to the control, after 8 days of pathogen inoculation (Table 1). “This treatment is not explained in the methods, it seems that they included it after the initial experiment; therefore, it is not explained or justified because they did it”.

Answer:
We have explained this treatment in the method in section 2.1. Laboratory assay of biological control of Fusarium crown and root rot of tomato, line 40-43: “An additional experiment using a living mycelial disk (3-mm diameter) without an enveloping membrane was also done. In this experiment, the inoculation period of the HBNR varied from 0 h to 12 h after inoculation with the pathogen and 0 h to 24 h prior to inoculation with the pathogen”.

14. At a distance of 6 cm, the reduction by HBNR W1 and Rhv7 was 71 % and 71 %, respectively. “For both”.

Answer:
We agree with your suggestion and we change and 71 %, respectively to be for both.

15. In the experiment using the WA system method, inoculation of the living HBNR mycelia on the base hypocotyls, and the pathogen on a different site 0, 3, 6, and 9 cm away from HBNR, showed that all HBNR isolates tested significantly reduced FCRR lesion development. Maximum protection occurred when the pathogen was inoculated at the position of 0 and 3 cm away. However, protection decreased a little bit at a distance of 6 and 9 cm. In this system, although the pathogen was directly introduced to the root surface, high lesion reduction was still provided by HBNR. Pre-inoculation of living mycelia of HBNR at a different time, and challenged with FORL, resulted in significant reduction in FCRR lesion development, even when HBNR was inoculated simultaneously (0 h) or 12 h after inoculation of pathogen.

Answer:
In this paragraph, results are presented, they are not conclusions

16. These results support those obtained by [21]: Rhizoctonia damping-off in bedding plants was still reduced when binucleate Rhizoctonia was applied together with R. solani AG-4 and AG-8. [22] also reported that application of Trichoderma harzianum Th-3013 was still able to control purple blotch disease even when performed 48 h after pathogen inoculation. However, a contrary result was reported by [23]. “Reference 21 and 23 very old”.

Answer:
We aggree with the suggestion, the paragraph was already deleted

17. tomato seedlings treated with non-pathogenic Fusarium 7 or 14 days before inoculation of the pathogen showed the greatest effect. However, the protective effect almost disappeared when both were applied simultaneously. The different results achieved by different researchers might be caused by a difference in the mechanisms of disease suppression involved in the varying system. “There is no discussion, they only make a comparison with other studies”.

Answer:
We aggree with the suggestion, the paragraph was already deleted.

18. Induced resistance in tomato plants by HBNR may be one of the mechanisms of biological control against FCRR in this study. “It is necessary to check it”.

Answer:

HBNR isolates used in the study did not shown any inhibition to FCRR pathogen *in vitro*, and whereas no contact occurred between HBNR isolates and the pathogen in our water agar rapid biocontrol assay, suggesting induced resistance operates as the biocontrol mechanism.

19. These results confirm those of [24] and [25], who reported that HBNR did not inhibit or parasitize R. solani. “Very old references. 31 years later, there is much more information regarding the subject”.

Answer:
We agree with your opinion, however, we did not find any recent experiment regarding antagonistic effect of Hypovirulent binucleate *Rhizoctonia* or non-pathogenic *Rhizoctonia*. Because it is already proved by:
[17]. Cardoso, JE. And E.Echandi. Nature of protection of bean seedlings from *Rhizoctonia* root rot by a binucleate *Rhizoctonia*-like fungus. Phytopathol 1987; 77 (11): 1548-1551. [http://doi.org/:10.1094/Phyto-77-1548].
[18]. Sneh, B., M.Ichielevich-Auster and I.Shomer. Comparative anatomy of colonization of cotton hypocotyls and roots by virulent and hypovirulent isolates of *Rhizoctonia solani*. Can J Bot 1989; 67 (7) 2142-2149. [http://doi.org/: 10.1139/b89-271].

20. Various bioassays for screening biocontrol agents use soil systems [14];[16];[31], and other bioassays for induced resistance in tomato plants have been reported, such as split root, benomyl, cutting, and layering [32]. However, these systems, like most other bioassays used for screening of biocontrol agents, often require more than one month to complete. Such long-term bioassays are difficult to use in large screening trials. In contrast, the bioassay used in this study offers the advantage of a short assay period (12 - 18 days) and requires only a small amount of space in cleanroom to test many different strains or isolates. Another advantage of this assay was its simplicity and the need for only small amounts of biocontrol agent and pathogen inoculum. By screening strains initially on plants, as opposed to pathogen-inhibition assays in Petri dishes, we hope to minimize the erroneous selection of strains on the basis of biological control traits that would not be expressed in more complex ecosystems. “This paragraph should be passed to the introduction, to justify the implementation of the assay they propose”.

Answer:
Thank you for reviewer suggestion. Paragraph to justify the implementation of the assay procedure had been included in Introduction. This paragraph is intended to compare with other biocontrol assay system

21. The results presented in this study establish that this rapid bioassay can be might also effectively to screen large numbers of microorganisms as biocontrol agents and for induce resistance activity. We expect that the bioassay used in this study could be also useful as a rapid assay in pathogenicity testing of FCRR. “Induce resistance activity or induced systemic resistance (ISR)? “

Answer:
We prefer to use induce resistance activity. Induce resistance activity by HNBR includes either ISR and SAR. Sharon M, Freeman S, Sneh B (2011) Assessment of Resistance Pathways Induced in Arabidopsis thaliana by Hypovirulent Rhizoctonia spp. Isolates. Phytopathology 101: 828–838.

22. In this experiment using the Water Agar system method, tomato seedlings treated with living mycelia, dead mycelia, and CF of HBNR isolates and Fusarium oxysporum f.sp. radicis-lycopersici (FORL) were inoculated at positions 0, 3, 6, and 9 cm away from the position of the HBNR resulted in significantly reduced lesion development of FCRR. The reduction of lesion development of FCRR decreased with the longer distance between HBNR and FORL. Pre-inoculation of living mycelia of HBNR at a different time, and challenged with FORL, resulted in significant reduction in FCRR lesion development, even when HBNR was inoculated simultaneously (0 h) or 12 h after inoculation of pathogen. “In this paragraph, results are presented, they are not conclusions”.

Answer:
We delete the paragragh and change as showed in the revised manuscripts

23. This method was also simple and least demanding of space and growth facilities. “It is not concluded about the objective (2): L1 (AG-Ba), W1, W7 (AG-A), and HBNR Rhv7”

Answer:
We Revised the conclusion as showed in the revised manuscripts.

24. Fig.(1). Diagram of laboratory assay of hypovirulent binucleate Rhizoctonia (HBNR) to suppress the disease development of Fusarium crown and root rot (FCRR) of tomato and to induce resistance against the disease, using the water agar method. (A) Inoculation point of HBNR consisting of a living mycelial disk (3-mm diameter), a dead mycelial disk (7-mm diameter), and CF (70 µl). In order to avoid direct contact between HBNR and FORL, the mycelial disk of living cells was enveloped by a polycarbonate membrane filter (0.2-µm mesh); (B) Inoculation point of Fusarium oxysporum f.sp. radicis-lycopersici (FORL) with spore suspension (5 µl of pathogen suspension at 5 × 105 spores/ml) at 0, 3, 6, and 9 cm away from the position of HBNR inoculum (separate experiment for each position); (C) Tomato root. “The scale of the figure is inadequate, the size of the root in relation to the leaf area does not correspond. What is the support of the seedling? “

Answer:
We have already reshape the size

25. Fig.(2). Effect of living mycelia of HBNR isolates on lesion development of Fusarium crown and root rot of tomato, after being challenge-inoculated with FORL at 0 cm (A), 3 cm (B), 6 cm (C), and 9 cm (D) away from the position of HBNR inoculum. Data are means of 4 replications with 5 seedlings per replication. "In Figure 3 the measurement was made on day 5, in this figure measurements are made from day 2 to 10, why the difference? It is the same system".

Answer:
For consistency of time for measurement of lesion development, we change all the results recorded until 8 days

26. Fig.(3). Effect of dead mycelia of HBNR isolates on lesion development of Fusarium crown and root rot of tomato, after being challenge-inoculated with FORL at 0 cm (A), 3 cm (B), and 6 cm (C) away from the position of HBNR inoculum. Data are means of 4 replications with 5 seedlings per replication. Data were recorded 5 days after pathogen inoculation. Bars labeled with the same letter are not significantly different according to Fisher's least significant different test ($P > 0.05$). "Why is this figure recorded on day 5?

Answer:
Because after day 5, the effect was almost disappear. So we did not show the data, eventhough we have the data.

27. Fig.(4). Effect of culture filtrates of HBNR isolates on lesion development of Fusarium crown and root rot of tomato after challenge-inoculation with FORL at 0 cm (A), 3 cm (B), and 6 cm (C) away from the position of HBNR inoculum. Data are means of 4 replications with 5 seedlings per replication. Bars represent standard error of the mean. "In figure 4 the measurements were made from day 2 to 8, in figure 2 they were made from 2 to 10. If it is the same system, why the differences in the taking of measurements?"

Answer:
We would like to know their ability in various distance and inoculated HBNR and FOR with difeferent inoculation point, in order to avoid direct contact between HBNR and FORL,

28. Fig (5). Effect of living mycelia of HBNR isolates covered with polycarbonate membrane filter (0.2-□m mesh) on lesion development of Fusarium crown and root rot of tomato after challenge-inoculation with FORL at 0 cm (A), 3 cm (B), and 6 cm (C) away from the position of HBNR inoculum. Data are means of 4 replications with 5 seedlings per replication. Bars labeled with the same letter are not significantly different according to Fisher's least significant different test ($P > 0.05$). "In Figure 5, why are only the results of HBNR W1 reported?"

Answer:
Yes, we just test representative the strongest isolate that is HBNR W1

29. Table 1. Effect of hypovirulent binucleate Rhizoctonia (HBNR) with various pre-incubation times on the reduction of lesion development of Fusarium crown and root rot (FCRR) of tomato caused by Fusarium oxysporum f. sp. radicis lycopersici (FORL) in water agar [a] Lesion development (cm)[b] "Why is reported in table 1 the size of the lesion in cm, and in the previous graphs in mm?. There was more lesion in this assay? Why are only results from W1 and Rhv7 reported?"

Answer:
The measurement scale has been changed to be in cm in the graphs

30. [a] Eight-day-old tomato seedlings were grown in 2 % water agar treated with HBNR and challenge-inoculated with FORL.
[b] Lesion development was recorded 8 days after inoculation with FORL.
[c] Inoculation points of FORL were 3 cm and 6 cm away from HBNR position.
[d] Pre-incubation of HBNR on neck root: 12 h after inoculation of pathogen (-12); simultaneous inoculation of HBNR and pathogen (0 h); 12 h before inoculation of pathogen (12); 24 h before inoculation of pathogen (24).
[e] Mean of four replications with five seedlings per replication. Values followed by the same letter do not differ significantly ($P > 0.01$) according to Fisher's least significant difference test. "The information must be included in the methods section".

Answer:

We have already explained this treatment in the method in section 2.1. Laboratory assay of biological control of Fusarium crown and root rot of tomato, line 40-43: "An additional experiment using a living mycelial disk (3-mm diameter) without an enveloping membrane was also done. In this experiment, the inoculation period of the HBNR varied from 0 h to 12 h after inoculation with the pathogen and 0 h to 24 h prior to inoculation with the pathogen".

A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate *Rhizoctonia* in Reducing Fusarium Crown and Root Rot of Tomato

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[3)] River Basin Research Center, Gifu University, Gifu 501-1193, Japan.

Abstract:

Background:
Fusarium oxysporum f. sp. *radicis-lycopersici* (FORL) caused Fusarium crown and root rot of tomato (FCRR), it's a serious constraint on tomato production and contributing to yield losses.

Aims/Method:
Using a rapid bioassay, hypovirulent binucleate *Rhizoctonia* (HBNR) were tested for their ability to reduce fusarium crown and root rot (FCRR) of tomato, caused by *Fusarium oxysporum* f.sp. *radicis lycopersici* (FORL). Roots of tomato seedlings growing on 2 % water agar in plastic boxes were inoculated with living or dead mycelial disks of HBNR. After 24 h, the pathogen was applied at 0, 3, 6, and 9 cm away from the position of the HBNR.

Result
When living HBNR was used, the treatments provided significant protection to tomato seedlings from FCRR infection at all distances tested. Tomato plants pre-inoculated with living HBNR at different times (12 h and 24 h before inoculation with the pathogen) and challenged with FORL showed significant reduction of FCRR lesion development. Significant reduction was still observed even when HBNR was inoculated simultaneously with or 12 h after inoculation of pathogen. Seedlings treated with dead HBNR and culture filtrates also showed significantly reduced FCRR lesion development. When living HBNR were enveloped by polycarbonate membrane filter, significant reduction of FCRR lesion development was still observed. In all experiments, reduction of FCRR lesion development in seedlings treated with HBNR tended to decrease with longer distance from the inoculation point of FORL and HBNR. We developed a simple, rapid, and miniaturized bioassay for evaluating the efficacy of HBNR against FORL. The bioassays require only 12 - 18 days, which is at least 12 days less than the soil system employed by previous researchers.

Keywords: ~~Hypovirulent Binucleate *Rhizoctonia*,~~ Non-pathogenic *Rhizoctonia*, *Fusarium oxysporum* f.sp. *radicis-lycopersici*, ~~Tomato, Rapid~~ Rapid Biocontrol~~Bio~~ A~~a~~ssay

Commented [E1]: Preferably, the keywords must be different from the words included in the title.

Commented [SS2R1]: We agree, we propose the keywords are: Non-pathogenic *Rhizoctonia*, *Fusarium oxysporum* f.sp. *radicis-lycopersici*, Rapid Biocontrol Assay

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1. INTRODUCTION

Fusarium crown and root rot of tomato (FCRR), caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), is a serious constraint on tomato production that limits the yield of ~~glasshouse~~greenhouse~~-~~ and field-grown tomato crops [1]. The disease was first detected in Japan in 1974 [2]. Yield losses caused by FCRR in greenhouse and field tomato production range from 15 to 65% ~~were 33 % and 44 % in Hokkaido and Kochi Prefectures, respectively~~ [3].

Recent research on the management of Fusarium wilt and FCRR has focused on diverse strategies, either individually or in combination. These strategies include host resistance and chemical, biological, and physical control [4]. Vitale *et al.* [5] demontrated that grafting tomato hybrid plants onto "Natalia" rootstock significantly enhanced the tolerance of plants to FORL, even ~~-~~though proteomic analysis showed a higher representation of proteins associated with pathogen infection. A combination of a plant-growth-promoting strain of *Fusarium equiseti* with biodegradable pots was also an effective control of FCRR [6].

~~Several studies have demonstrated that *Pseudomonas* sp. strain FC-24B, *P. putida* FC-8B [8] and *P. chlororaphis* [9] effectively reduced *Fusarium oxysporum* f. sp. *radicis-lycopersici*. In a study using four rhizospheric bacteria (*Bacillus*, *Lysinibacillus*, *Enterobacter*, and *Serratia*) and one root-associated endophytic (RAE) associated with *Alcaligenes faecalis* subsp. caused a statistically significant decrease in plant infection by FORL through antibiosis mechanisms [10]. [11] reported that *Pseudomonas fluorescens* WCS365 and *P. chlororaphis* PCL1391 effectively controlled FCRR through induced systemic resistance.~~

~~———~~Hypovirulent binucleate *Rhizoctonia* (HBNR) were investigated as effective biocontrol agents for a number of important diseases caused by *Rhizoctonia solani* [7] and~~–~~ *Phytium* [8]. Our previus research showed that HBNR effectively controls Fusarium wilt of tomato [9], Fusarium wilt of spinach [10], and Fusarium crown and root rot of tomato [11]. These studies indicated that one of the mechanisms of biocontrol of fusarium diseases with HBNR isolates might be induced resistance. Investigations of HBNR as an agent of induced systemic resistance (ISR) in beans, against the root rot pathogen *Rhizoctonia solani* or the anthracnose pathogen *C. lindermuthianum*, have also been reported [12]. HBNR also effectively protected cotton seedlings against rhizoctonia damping-off and alternaria leaf spot with mechanism of induced systemic resistance (ISR) [13].

A major limiting factor in the development of biological control strategies for different plant diseases is the formulation of efficient procedures for rapidly screening large numbers of organisms for biological control activity. While field screening should theoretically provide the best detection of efficient biocontrol strains, limitations of space, labor, cost, and optimal environmental conditions preclude the use of this type of screening strategy. Laboratory assays based on the *in vitro* inhibition of pathogens or production of particular metabolites by biological control agents offer a rapid and relatively inexpensive means of screening organisms but may not be good indicators of biocontrol potential. Unsurprisingly, biocontrol strains selected *in vitro* on the basis of phenotypes with unknown links to biological control activity in plant systems do not always perform as expected under greenhouse or field conditions [14 ~~],[~~- 15]. The present study was undertaken to: (1) develop a rapid and miniaturized laboratory bioassay for screening the efficacy of HBNR in reducing

Commented [E3]: Greenhouse

Commented [SS4R3]: We agree to change glasshouse to be Greenhouse

Commented [E5]: Include more recent studies to justify the losses in tomato production.

Commented [SS6R5]: We include the recent study: Yield losses due to FCRR in greenhouse and field tomato production range from 15 to 65% (Ozbay and Newman 2004).

FCRR in the tomato; (2) investigate the efficacy of various inoculum forms (living and dead mycelial disks) of HBNR in controlling FCRR using a water agar system.

2. MATERIALS AND METHODS

Organisms: Four isolates of HBNR were used as biocontrol agents: L21 (AG-Ba), W1, W7 (AG-A), and HBNR Rhv7 (unknown anastomosis group). *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) isolate RJNI, obtained from a tomato infested with fusarium crown and root rot (FCRR), was used as the inoculum of the pathogen.

Plant: Tomato cv. "House Momotaro", a popular cultivar that is susceptible to FCRR, was used throughout the experiments.

Inoculum preparation: (1) The pathogen, FORL, was grown on potato dextrose agar (PDA) for 7 days in the dark at 25 °C. Spores were scraped from the cultures with a sterile glass bar, and a spore suspension was prepared in sterile water and filtered through eight layers of sterile gauze. (2) HBNR isolates were prepared as inoculum forms in potato dextrose agar (PDA) plugs (living and dead mycelial disks). The isolates were grown on PDA for 3-7 days in the dark at 25 °C. The dead mycelial disk was prepared by killing the 7-day-old culture with chloroform and then drying it for 60 min on a clean bench. To make culture filtrate (CF), two mycelial disks of each HBNR isolate, obtained from the growing margin of a colony on PDA, were transferred to a 200-ml flask containing 50 ml of potato dextrose broth (pH 6.5). The isolates were cultured without shaking for 10 days in dark. The crude culture filtrate was separated from mycelia and filtered three times through three layers (each time) of Whatman no. 2 filter paper. The CF was then filter sterilized (0.45-µm Millipore filters, Millipore Products Division, Bedford, USA).

2.1. Laboratory assay of biological control of Fusarium crown and root rot of tomato

The efficacy of HBNR in suppressing the development of FCRR in the tomato was tested in laboratory experiments using a water agar (WA) system method (Fig. 1). Tomato seeds were surface-sterilized in 70 % ethyl alcohol for 1 min followed by soaking in 1 % sodium hypochlorite with 3 drops of Tween 20 (polyoxyethylene sorbitan monolaureate; Nacalai Tesque, Inc., Kyoto, Japan) for 20 min. The seeds were then rinsed three times with sterilized distilled water (SDW). The seeds were pre-germinated on 2 layers of Whatman No. 1 filter paper for 3 days in the dark at 25 °C. Five seedlings were transferred to a sterilized plastic box (196 × 104.5 × 28 mm) containing water agar (WA) and allowed to grow for 6 days at about 20 in a cleanroom. A living HBNR mycelial disk (3-mm diameter, taken from the advancing margin of a three-day-old culture), a dead mycelial disk (7-mm diameter), and CF (70 µl) were used to inoculate the basal hypocotyls of the seedlings, which were again incubated for 24 h. To prevent spread and maintain a uniform distribution of CF on basal hypocotyls or roots, drops of CF were placed on an 8-mm diameter paper disc with 1.5-mm thickness (Advantec, Toyo Roshi Kaisha, Ltd. Japan). To avoid direct contact between HBNR and FORL, the mycelial disk of HBNR was enveloped by a polycarbonate membrane filter (0.2-µm mesh). An additional experiment using a living mycelial disk (3-mm diameter) without an enveloping membrane was also done. In this experiment, the inoculation period of the HBNR varied from 0 h to 12 h after inoculation with the pathogen and 0 h to 24 h prior to inoculation with the pathogen.

Commented [E7]: According to the background, it is already proven that HBNR controls FCRR.

Commented [SS8R7]: We agree that HBNR have been proven effectively control FCRR according to the background. Since we develop a rapid method to evaluate HBNR against FCRR in laboratory assay in particular for selecting a large number of HBNR, therefore it is still necessary to mention [develop a rapid and miniaturized laboratory bioassay for screening the efficacy of HBNR in reducing FCRR in the tomato] as our objective.

Commented [E9]: Write what are the inoculum forms used.

Commented [SS10R9]: We agree the inoculum forms used to be included in our manuscript. We rewrite the objective no 2 to be (2) investigate the efficacy of various inoculum forms (living and dead mycelial disks) of HBNR in controlling FCRR using a water agar system

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Commented [E11]: Explain the differences of each HBNR isolate.

Commented [SS12R11]: We have already explained the differences of isolate in the parentheses such as, L2 (AG-Ba) means that L2 belong to the anastomosis group Ba.

Commented [E13]: According to the method used, it is not a simple and rapid bioassay, nor is it cheap.

Commented [SS14R13]: When we compare to the ordinary evaluation of HBNR against plant disease in greenhouse which is need seedling preparation (21 days) in small pot then were transferred to bigger plastic pot containing pathogen-infested soil medium for recording of disease severity for about 70 days. In this study we just need cheap materials (plastic box and water agar) and we just need only nine days for seedling preparation and ten days for diseases recording.

Commented [E15]: Why try the dead mycelium? Explain.

Commented [SS16R15]: Beside living cell of antagonist use as biocontrol agent, dead cell such as dead mycelium also can be used as effective biocontrol agent as reported by:
Zhang, H.J., Dong, H.Z., and Li, W.J. 2011. Dead mycelium of *Penicillium chrysogenum* protects transplanted cotton plants against fungal wilts in a saline field. Spanish Journal of Agricultural Research 9(3): 873-881.

As a control, seedlings were inoculated with HBNR-free PDA or SDW. Then, 5 µl of pathogen suspension (5 × 10^5 spores/ml) were inoculated at positions 0, 3, 6, and 9 cm away from the position of the HBNR inoculum. A 5-mm diameter disk of lens paper was placed on each drop to prevent runoff and to maintain a uniform distribution of spores on the root surface. The treatments were prepared in four replicates. Treated and control seedlings were maintained at about 20 °C for another 2-8~~10~~ days. Disease severity was determined by measuring lesion development at the pathogen inoculation point. Percent reduction of lesion development was used to measure the efficacy of HBNR against the pathogen, by employing the formula [(A-B)/A] ×~~x~~ 100, in which A represents the lesion length observed on the root due to inoculation of pathogen alone and B is the lesion length observed on the root due to co-inoculation of HBNR and the pathogen.

2.2 Data analysis

The experiments were carried out in completely randomized design. Treatment means obtained for lesion development of FCRR were compared using Fisher's least significant difference (LSD) test with critical values of $P = 0.05$.

3. RESULTS

3.1 Biological control of FCRR of tomato with HBNR

In a WA system, tomato seedlings treated with living mycelia, dead mycelia, and CF of HBNR isolates significantly reduced lesion development of FCRR ($P = 0.05$).

When living mycelia were used as treatment, seedlings treated with HBNR isolates had significantly less FCRR lesion development after 4-8~~10~~ days of pathogen inoculation (~~$P = 0.01$;~~ Fig. 2). The percentage of reduction tend to decrease with the longer distance between HBNR and FORL. At a distance of 0 cm between HBNR and FORL, the reduction of lesion development by HBNR L2~~2~~, W1, W7, and Rhv7 was almost completely ranged from 8~~81~~-9~~86~~ %. At a distance of 3 cm, application of all HBNR still higly reduced lesion development by 8~~872~~-9~~61~~ %. At a distance of 6 cm and 9 cm, the reduction of lesion development by all HBNR isolates slightly decreased by 5~~525~~-9~~8~~4 % and 1~~135~~-6~~675~~ %, respectively (Fig. 2).

Tomato seedlings treated with dead mycelia of all HBNR isolates except L2 also showed significant reduction of FCRR lesion development ~~5~~2-8 days after inoculation with the pathogen (~~$P = 0.05$;~~ Fig. 3). At a distance of 0 cm, lesion development reduction was 6-21%, 22-79%, 9-49%, and 4-52%, ~~19 %, 62 %, 41 %, and 30 %~~ for HBNR L2, W1, W7, and Rhv7, respectively (Fig. 3A). At a distance of 3 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 5-37%, 16-52%, 10-41%, and 9-59%, ~~18 %, 52 %, 32 %, and 34 %,~~ respectively (Fig. 3B). At a distance of 6 cm, lesion development reduction was 2-34%, 15-45%, 10-49%, and 4-48%, ~~21 %, 38 %, 42 %, and 32 % for HBNR L2, W1, W7, and Rhv7,~~ respectively (Fig. 3C).

The application of CF of HBNR isolates also resulted in ~~significant reduction~~significant reduction in FCRR lesion development 2-8 days after pathogen inoculation ($P = 0.05$; Fig. 4). At a distance of 0 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 35-8~~5100~~ %, 36-~~100~~73 %, 37-~~10064~~%, and 36-~~10078~~%, respectively (Fig. 4A). At a distance of 3 cm,

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Commented [E17]: How was the lesion size measured? Did they only use a ruler, or was a vernier used? It is very important, because the work is based on the results of the measurement of the lesion.

Commented [SS18R17]: Lesion length was measured using a Vernier caliper

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Commented [E19]: In the methods it appears as L1.

Commented [SS20R19]: We apologize for mistyping L2 in the Methods. The correct one is L1

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Commented [E21]: Why do they mention that there was a 100% reduction of lesion? If, on day 2, the disease just begins to develop, it is not controlled.

Commented [SS22R21]: Thank you very much for your excellent suggestion. We have rewrite with the percentage lesion reduction from day 4th.

At a distance of 0 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 35 – 85 %, 36 – 73 %, 37 - 64%, and 36 - 78%, respectively (Fig. 4A). At a distance of 3 cm, treatment with HBNR L2, W1, W7, and Rhv7 reduced lesion development by 30 – 79 %, 31 – 83 %, 23 – 74 %, and 27 - 88%, respectively (Fig. 4B). At a distance of 6 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 30 – 70 %, 33 – 72 %, 26 – 84 %, and 27 – 86 %, respectively (Fig. 4C). "Why do they mention that there was a 100% ...

Commented [SS23R21]:

treatment with HBNR L2, W1, W7, and Rhv7 reduced lesion development by 30–87 79 %, 31–100 83 %, 22 23–100 74 %, and 27–10088%, respectively (Fig. 4B). At a distance of 6 cm, the reduction of lesion development by HBNR L2, W1, W7, and Rhv7 was 20 30–70 %, 33–100 72 %, 26–100 84 %, and 27–100 86%, respectively (Fig. 4C).

We attempted to prevent direct contact between HBNR and FORL by enveloping the living mycelia in a polycarbonate membrane filter (0.2-µm mesh), but the mycelia still penetrated the membrane, so that direct contact between HBNR and FORL was observed. In this experiment, significant reduction in FCRR lesion development was still observed up to 8 days after pathogen inoculation at a distance of 0 cm (P = 0.05; Fig. 5A). At a distance of 3 cm, significant reduction in FCRR lesion development was observed until 6 days after pathogen inoculation still observed 4–6 days after pathogen inoculation at a distance of 0–3 cm (P = 0.05; Fig. 5A, 5B). However, at a distance of 6 cm, significant reduction was only observed at 3-4 days after pathogen inoculation (Fig. 5C). The reduction of lesion development by HBNR W1 was 25-78%, 13-67%, and 10-52% 34–61 %, 45–57 %, and 2–36 % at distances of 0, 3, and 6 cm, respectively.

In another experiment, pre-inoculation at 12 h and 24 h with living mycelia of HBNR W1 or Rhv7 on the seedlings, and challenge-inoculation with FORL at 3 cm and 6 cm away from HBNR, also resulted in significant reduction in lesion development compared to the control, after 8 days of pathogen inoculation (Table 1). At 12 h pre-inoculation of HBNR, at a distance of 3 cm, treatment with HBNR W1 and Rhv7 reduced FCRR lesion development by 90-% and 91-%, respectively. At a distance of 6 cm, the reduction by HBNR W1 and Rhv7 was 71 % for bothand 71 %, respectively. The reduction slightly increased with the longer pre-inoculation period of 24 h. At a distance of 3 cm, the reduction by HBNR W1 and Rhv7 was 93-% and 90-%, respectively. At a distance of 6 cm, the reduction by HBNR W1 and Rhv7 was 82–% and 74–%, respectively. HBNR isolates also significantly reduced lesion development of FCRR (P = 0.01) when both isolates were applied simultaneously (0 h) and even when HBNR was applied 12 h after pathogen inoculation. At 0 h, or simultaneous inoculation, at a distance of 3 cm, the reduction of lesion development by HBNR W1 and Rhv7 was 89-% and 90-%, respectively. At a distance of 6 cm, the reduction was 71-% and 64-% for HBNR W1 and Rhv7, respectively. At 12 h after pathogen inoculation, at a distance of 3 cm, the reduction was 89-% and 81-% for HBNR W1 and Rhv7, respectively. At a distance of 6 cm, the reduction by HBNR W1 and Rhv7 was 66-% and 59-%, respectively.

4. DISCUSSION

In this study, all HBNR isolates tested using various inoculum forms, i.e. living mycelia, CF, and dead mycelia significantly reduced lesion development of FCRR. Maximum protection occurred when the pathogen was inoculated at the position of 0 and 3 cm away. However, protection decreased at a distance of 6 and 9 cm. In our study using the WA system method, the phenomena lesion development affected by biological control agents could be rapidly recorded without destructive to the root system. Living mycelia showed a stronger inhibition of lesion development throughout experiment, while dead mycelium inhibited effectively lesion development up to 5 days then decrease at a longer time of incubation. It might be that on living mycelia, three were a competition in infection site between HBNR and FORL. HBNR has been reported to be an effective colonization of plant root [11], [16] and it was likely that inoculated living

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HBNR mycelia had been already colonizing the infection site that allow competition between HBNR and FORL. Pre-inoculation of living mycelia of HBNR at a different time, and challenged with FORL, resulted in significant reduction in FCRR lesion development, even when HBNR was inoculated simultaneously (0 h) or 12 h after inoculation of pathogen.~~In the experiment using the WA system method, inoculation of the living HBNR mycelia on the base hypocotyls, and the pathogen on a different site 0, 3, 6, and 9 cm away from HBNR, showed that all HBNR isolates tested significantly reduced FCRR lesion development. Maximum protection occurred when the pathogen was inoculated at the position of 0 and 3 cm away. However, protection decreased a little bit at a distance of 6 and 9 cm. In this system, although the pathogen was directly introduced to the root surface, high lesion reduction was still provided by HBNR. Pre-inoculation of living mycelia of HBNR at a different time, and challenged with FORL, resulted in significant reduction in FCRR lesion development, even when HBNR was inoculated simultaneously (0 h) or 12 h after inoculation of pathogen. These results support those obtained by [21]: *Rhizoctonia* damping off in bedding plants was still reduced when binucleate *Rhizoctonia* was applied together with *R. solani* AG 4 and AG 8. [22] also reported that application of *Trichoderma harzianum* Th 3013 was still able to control purple blotch disease even when performed 48 h after pathogen inoculation. However, a contrary result was reported by [23] tomato seedlings treated with non pathogenic *Fusarium* 7 or 14 days before inoculation of the pathogen showed the greatest effect. However, the protective effect almost disappeared when both were applied simultaneously. The different results achieved by different researchers might be caused by a difference in the mechanisms of disease suppression involved in the varying system.~~

Tomato seedlings treated with CF and dead mycelia of HBNR effectively reduced FCRR lesion development. The *in vitro* interaction experiments using living or dead mycelia and CF reveal that they did not produce any zone of inhibition (data not shown), suggesting that they were not antagonistic and ruling out the possible involvement of toxins or antifungal compounds in disease suppression. Since CF and dead mycelia of HBNR application sites and pathogen application sites were spatially separated by a distance of 3-6 cm, and there was no contact between HBNR isolates and the pathogen until day 5 at 3 cm and day 8 at 6 cm, we observed that average mycelial growth of the pathogen was 0.54 cm/day. Induced resistance in tomato plants by HBNR may be one of the mechanisms of biological control against FCRR in this study. These results confirm those of [~~24~~17] and [~~25~~18], who reported that HBNR did not inhibit or parasitize~~—~~ *R. solani*. Plant protection by hypovirulent binucleate *Rhizoctonia* involves resistance pathways such as systemic acquired resistance (SAR), induced systemic resistance (ISR), and phytoalexins [16].

Many reports demonstrated that mycelia or CF of fungi were effective in inducing resistance against various diseases [~~26~~19];[27];[28]. [- ~~29~~22] demonstrated that tomato plants treated with ~~Oligandrin~~oligandrin, the elicitin-like protein produced by the mycoparasite *Pythium oligandrum*, showed significant induction of systemic resistance against FORL. The most striking features of the resistance mechanism involved restriction of fungal growth to the outer root tissues, decrease in pathogen viability, and formation of aggregated deposits, which often accumulated at the surface of invading hyphae. In addition, [~~30~~23] reported that cucumber seedlings treated with pectinases extracted from fermentation products of *Penicillium oxalicum* BZH-2002 induced resistance against scab caused by *Cladosporium cucumerinum*.

Various bioassays for screening biocontrol agents use soil systems [~~14~~9,];[~~16~~11,];[~~31~~24], and other bioassays for induced resistance in tomato plants

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have been reported, such as split root, benomyl, cutting, and layering [~~32~~25]. However, these systems, like most other ~~bioassays~~ biocontrol assay~~used for screening of biocontrol agents~~, often require more than one month to complete. Such long-term bioassays are difficult to use in large screening trials. In contrast, the bioassay used in this study offers the advantage of a short assay period (12–~~–~~18 days) and requires only a small amount of space in cleanroom to test many different strains or isolates. Another advantage of this assay was its simplicity and the need for only small amounts of biocontrol agent and pathogen inoculum. By screening strains initially on plants, as opposed to pathogen-inhibition assays in Petri dishes, we hope to minimize the erroneous selection of strains on the basis of biological control traits that would not be expressed in more complex ecosystems.

The results presented in this study establish that this rapid bioassay can be might also effectively to screen large numbers of microorganisms as biocontrol agents and for induce resistance activity. We expect that the bioassay used in this study could be also useful as a rapid assay in pathogenicity testing of FCRR.

CONCLUSIONS

~~In this experiment using the Water Agar system method, tomato seedlings treated with living mycelia, dead mycelia, and CF of HBNR isolates and *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) were inoculated at positions 0, 3, 6, and 9 cm away from the position of the HBNR resulted in significantly reduced lesion development of FCRR. The reduction of lesion development of FCRR decreased with the longer distance between HBNR and FORL. Pre-inoculation of living mycelia of HBNR at a different time, and challenged with FORL, resulted in significant reduction in FCRR lesion development, even when HBNR was inoculated simultaneously (0 h) or 12 h after inoculation of pathogen.~~ The ~~Laboratory~~ laboratory assay developed in this study could be rapidly determined biocontrol efficacy of HBNR against FCRR within 12-18 days from seedling emergence. Except isolate L2, all isolates exhibited a strong and consistent biocontrol efficacy. ~~markedly shortened the time needed for evaluating the ability of HBNR to control FCRR. This assay require only 12 – 18 days from seedling appearance to rating for disease severity, which is at least 12 days less than the soil system employed by previous researchers. This method was also simple and least demanding of space and growth facilities.~~ Living mycelia were the most effectively used as a biocontrol inoculum, followed by CF, and dead mycelia.

ACKNOWLEDGMENTS

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Sharon M, Freeman S, Sneh B (2011) Assessment of Resistance Pathways Induced in Arabidopsis thaliana by Hypovirulent Rhizoctonia spp.
Isolates. Phytopathology 101: 828–838.

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[http://doi.org/10.1007/s12600-012-0271-z].
[22] Benhamou N, Bélanger RR, Reyand P, Tirilly Y. Oligandrin, the elicitin-like protein produced by the mycoparasite *Pythium oligandrum*, induces systemic resistance to *Fusarium* crown and root rot in tomato plants. Plant Physiol Biochem 2001; 39(7-8): 681-98.
[http://doi.org/10.1016/S0981-9428(01)01283-9].
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[http://doi.org/10.1007/BF02979849].
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[http://doi.org/10.1111/j.1365-3059.1995.tb02750.x]
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[http://doi.org/10.1094/PDIS.1997.81.5.492].

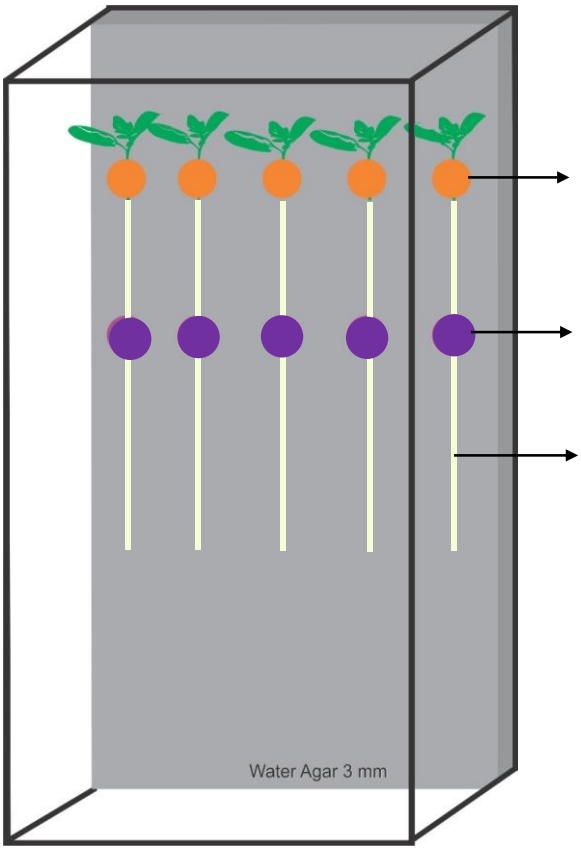
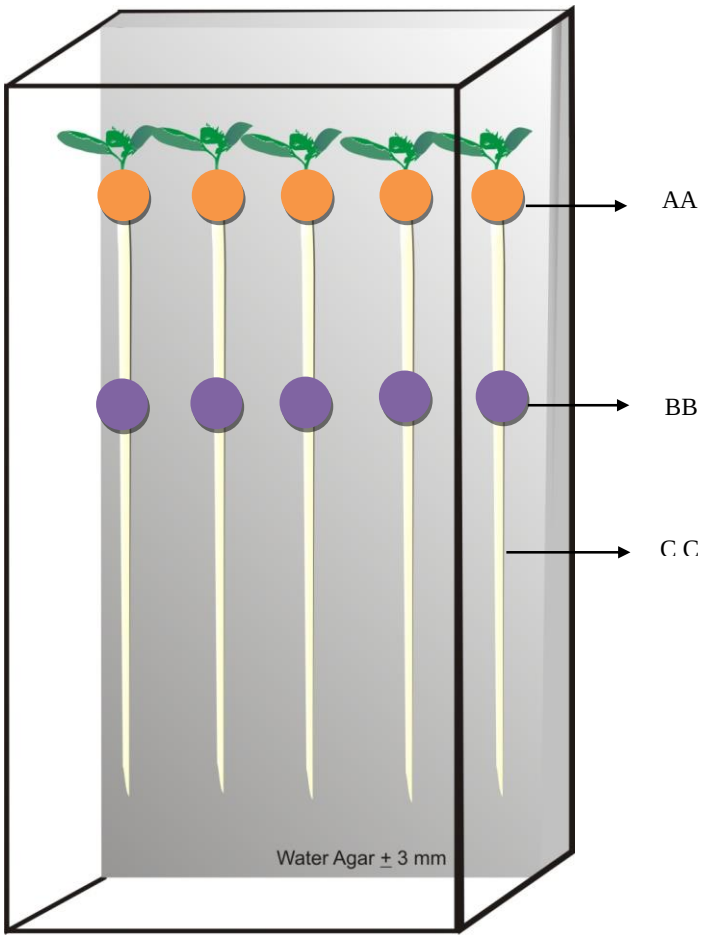


Fig. (1). Diagram of laboratory assay of hypovirulent binucleate *Rhizoctonia* (HBNR) to suppress the disease development of Fusarium crown and root rot (FCRR) of tomato and to induce resistance against the disease, using the water agar method. (**A**) Inoculation point of HBNR consisting of a living mycelial disk (3-mm diameter), a dead mycelial disk (7-mm diameter), and CF (70 µl). In order to avoid direct contact between HBNR and FORL, the mycelial disk of living cells was enveloped by a polycarbonate membrane filter (0.2-µm mesh); (**B**) Inoculation point of *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL) with spore suspension (5 µl of pathogen suspension at 5×10^5 spores/ml) at 0, 3, 6, and 9 cm away from the position of HBNR inoculum (separate experiment for each position); (**C**) Tomato root.



Commented [E39]: The scale of the figure is inadequate, the size of the root in relation to the leaf area does not correspond. What is the support of the seedling?

Commented [SS40R39]: We have already reshape the size.

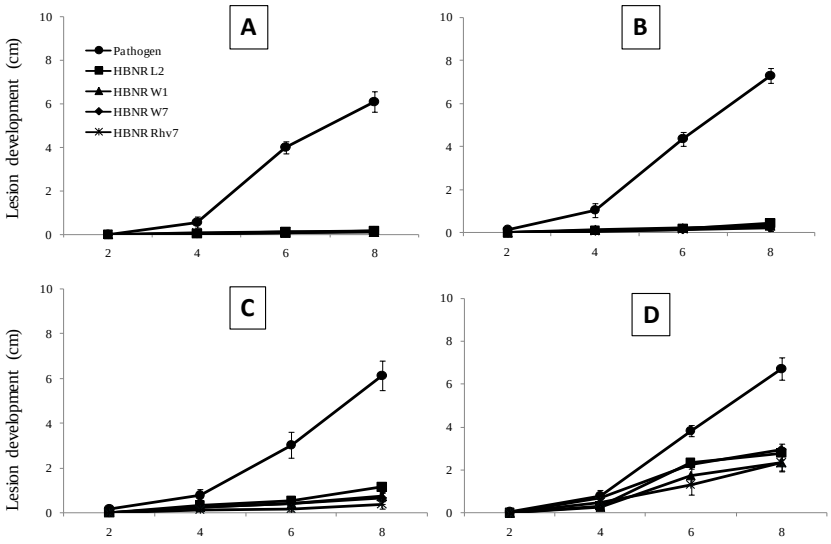
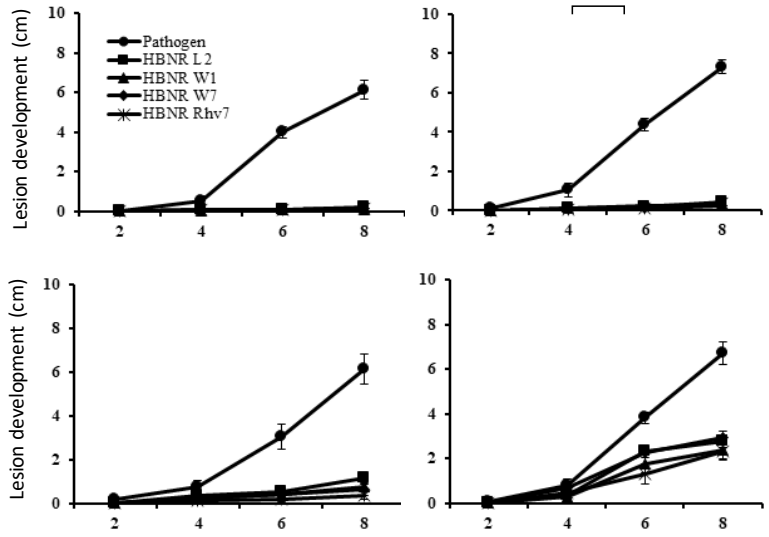

A
B
C
D
Pathogen
HBNR L2
HBNR W1
HBNR W7
HBNR Rhv7
Lesion development (cm)
Lesion development (cm)
Days after inoculation of pathogen

Fig. (2). Effect of living mycelia of HBNR isolates on lesion development of Fusarium crown and root rot of tomato, after being challenge-inoculated with FORL at 0 cm (**A**), 3 cm (**B**), 6 cm (**C**), and 9 cm (**D**) away from the position of HBNR inoculum. Data are means ± SEM of 4 replications with 5 seedlings per replication.



Commented [E41]: In Figure 3 the measurement was made on day 5, in this figure measurements are made from day 2 to 10, why the difference? It is the same system.

Commented [SS42R41]: For consistency of time for measurement of lesion development, we change all the results recorded until 8 days.

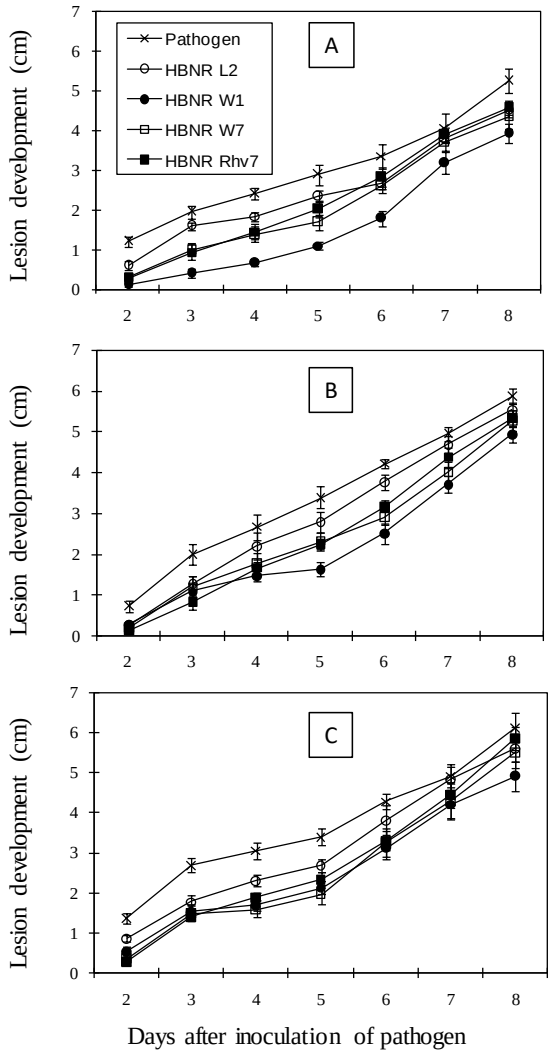


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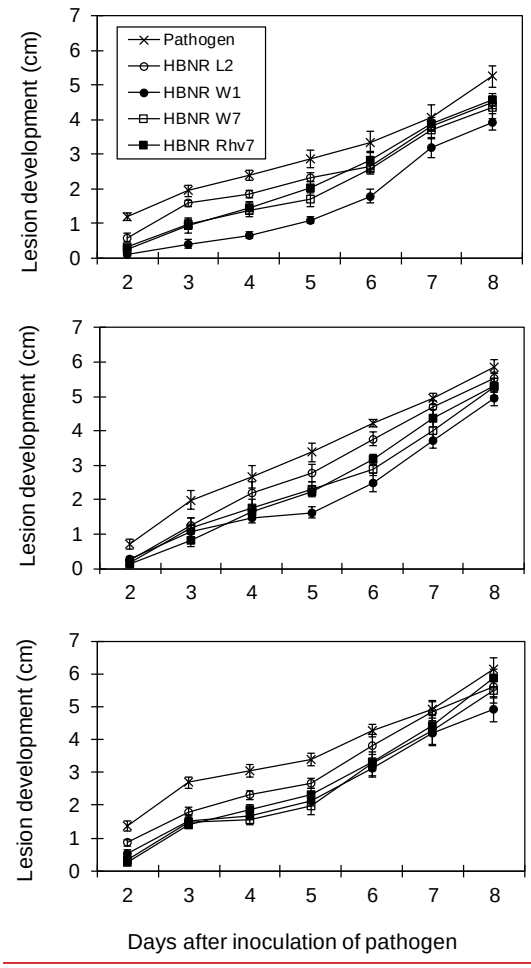
Fig. (3). Effect of dead mycelia of HBNR isolates on lesion development of Fusarium crown and root rot of tomato, after being challenge-inoculated with FORL at 0 cm (**A**), 3 cm (**B**), and 6 cm (**C**) away from the position of HBNR inoculum. Data are means ± SEM of 4 replications with 5 seedlings per replication. ~~Data were recorded 5 days after pathogen inoculation. Bars labeled with the same letter are not significantly different according to Fisher's least significant different test ($P > 0.05$).~~


Pathogen
HBNR L2
HBNR W1
HBNR W7
HBNR Rhv7
Lesion development (cm)
Lesion development (cm)
Lesion development (cm)
0
1
2
3
4
5
6
7
2
3
4
5
6
7
8
Days after inoculation of pathogen

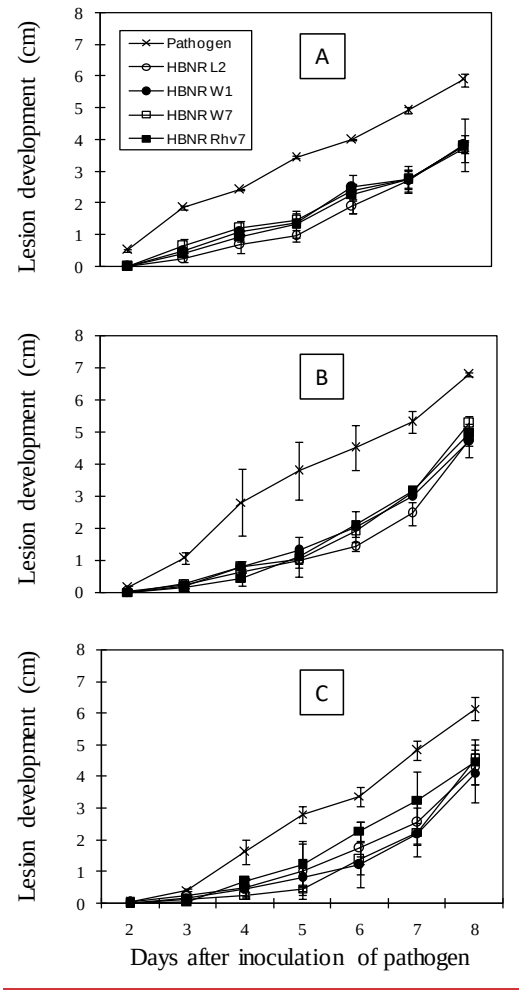
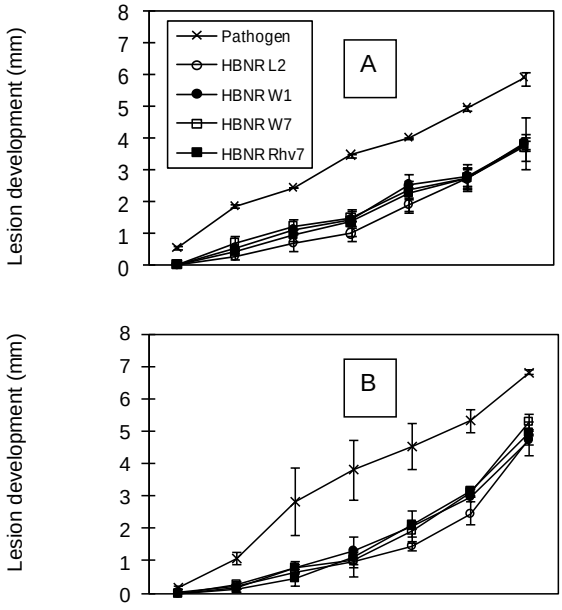


Fig. (4). Effect of culture filtrates of HBNR isolates on lesion development of Fusarium crown and root rot of tomato after challenge-inoculation with FORL at 0 cm (**A**), 3 cm (**B**), and 6 cm (**C**) away from the position of HBNR inoculum. Data are means ± SEM of 4 replications with 5 seedlings per replication. ~~Bars represent standard error of the mean.~~



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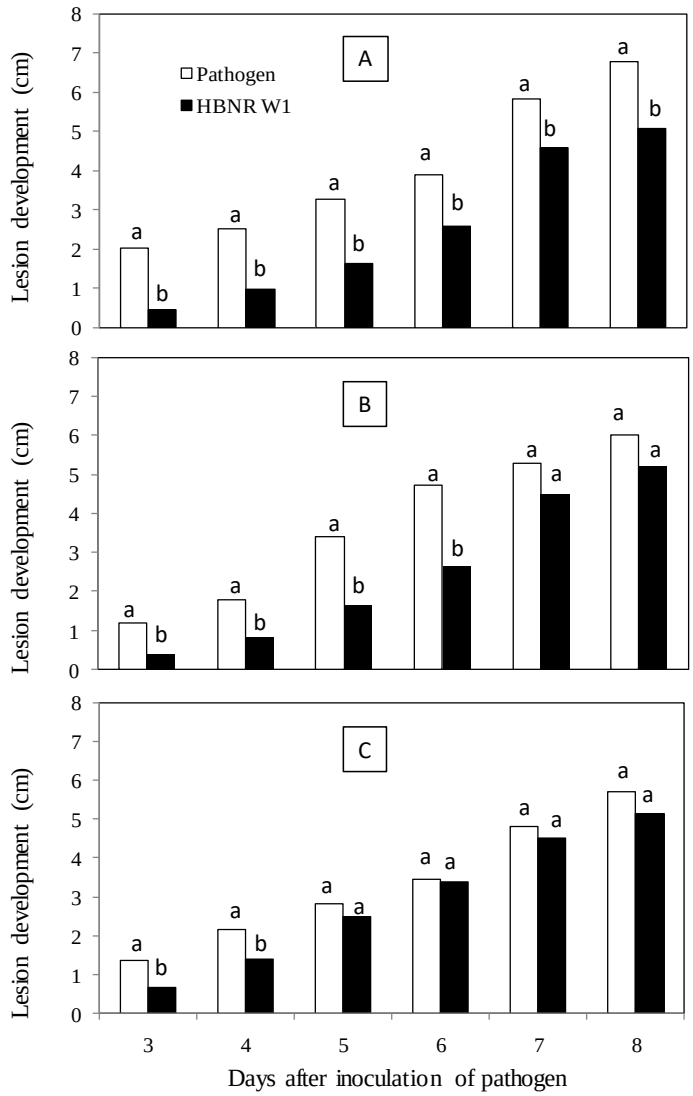

Fig. (5). Effect of living mycelia of HBNR isolates covered with polycarbonate membrane filter (0.2-µm mesh) on lesion development of Fusarium crown and root rot of tomato after challenge-inoculation with FORL at 0 cm (**A**), 3 cm (**B**), and 6 cm (**C**) away from the position of HBNR inoculum. Bars pathogen and HBNR W1 l~~Data are means of 4~~abelled with different letter ~~replications with 5 seedlings per replication~~are significantly different at $P<0.05$ according to Fisher's least significant difference test.~~ Bars labeled with the same letter are not significantly different according to Fisher's least significant different test ($P > 0.05$).~~

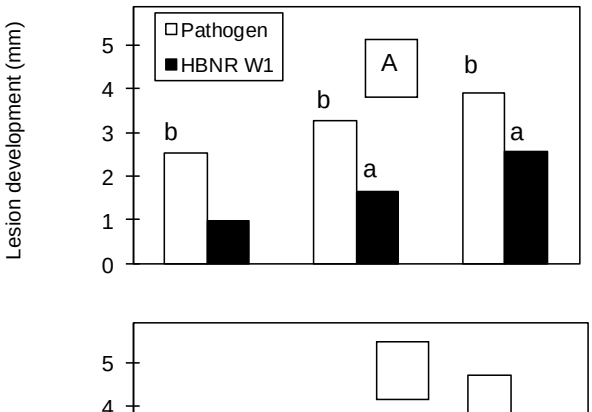

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Table 1. Effect of hypovirulent binucleate *Rhizoctonia* (HBNR) with various pre-incubation times on the reduction of lesion development of Fusarium crown and root rot (FCRR) of tomato caused by *Fusarium oxysporum* f. sp. *radicis lycopersici* (FORL) in water agar [a]

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Treatments	Lesion development (cm)[b]							
	3 cm[c]				6 cm			
	-12[d]	0	12	24	-12	0	12	24
Pathogen	7.2 b[e]	7.0 b	6.7 b	7.0 b	6.4 b	6.1 b	5.6 b	6.1 b
HBNR W1	0.8 a	0.8 a	0.7 a	0.5 a	2.2 a	1.8 a	1.6 a	1.1 a
HBNR Rhv7	1.4 a	0.7 a	0.6 a	0.7 a	2.6 a	2.2 a	1.6 a	1.6 a

[a] Eight-day-old tomato seedlings were grown in 2 % water agar treated with HBNR and challenge-inoculated with FORL.
[b] Lesion development was recorded 8 days after inoculation with FORL.
[c] Inoculation points of FORL were 3 cm and 6 cm away from HBNR position.
[d] Pre-incubation of HBNR on neck root: 12 h after inoculation of pathogen (-12); simultaneous inoculation of HBNR and pathogen (0 h); 12 h before inoculation of pathogen (12); 24 h before inoculation of pathogen (24).
[e] Mean of four replications with five seedlings per replication. Values followed by the same letter do not differ significantly ($P > 0.~~01~~05$) according to Fisher's least significant difference test.

Commented [E43]: Why is reported in table 1 the size of the lesion in cm, and in the previous graphs in mm?. There was more lesion in this assay? Why are only results from W1 and Rhv7 reported?

Commented [SS44R43]: The measurement scale has been changed to be in cm in the graphs

Commented [E45]: The information must be included in the methods section.

Commented [SS46R45]: We have already explained this treatment in the method in section 2.1. Laboratory assay of biological control of Fusarium crown and root rot of tomato, line 40-43: "An additional experiment using a living mycelial disk (3-mm diameter) without an enveloping membrane was also done. In this experiment, the inoculation period of the HBNR varied from 0 h to 12 h after inoculation with the pathogen and 0 h to 24 h prior to inoculation with the pathogen".

8. PERMINTAAN EDITOR UNTUK PERBAIKAN GRAFIK

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

TOASJ :: Query Regarding Graphics Enhancement

3 messages

Bentham Open - Sumaiya Azhar <sumaiya@benthamopen.com>
To: a_muslim@unsri.ac.id
Cc: editorial@benthamopen.org

Mon, Nov 19, 2018 at 1:19 PM

Query Regarding Graphics Enhancement

November 19, 2018

Ref # 63422

Dear Dr. Muslim,

Thank you for submitting your manuscript entitled,**"A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato "** for possible publication in **"The Open Agriculture Journal "**. During graphics assessment, it has been observed that the figure(s) provided in your article have not been provided according to our requirements (which are given below for your convenience) and are not of the required quality, making the text and the graphics indistinct on reproduction or on attempting to adjust the figure(s) to the specified width.

You are therefore requested to send better quality figure(s) **2,** in PDF, PPT, MS Word, TIFF or JPG versions. Please have these figures improved either, yourself or by professional graphic designers that may be in your organization/ country. If you do not have access to such facilities then you may also consider approaching our contracted service providers Eureka Science, for this.

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Technical requirements for graphic/ figure submissions.

- Submit the original artwork or a photographic print of the original for publication.
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- Format & Resolution: The following file formats can be accepted (our preference in order of appearance) : PDF, PPT, MS Word, TIFF or JPG
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Whenever possible, submit graphics that do not have to be reduced to fit the standard figure size. And, use the best resolution available.

Please do not hesitate to contact me if you have any query or you need any assistance from our end.

Looking forward to your response in due course.

With best regards,

S. Alavi

Manager Graphics

Bentham OPEN

editorial@benthamopen.org

For complaints contact: complaint@benthamopen.net

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a. muslim unsri <a_muslim@unsri.ac.id> Tue, Nov 20, 2018 at 2:20 PM
To: sumaiya@benthamopen.com

Dear S. Alavi,

Thank you very much for your suggestion. We have already enhanced figure 2 according to your suggestion. Please find the revised figure 2 in the attachment file.

Best regards,

A. Muslim

[Quoted text hidden]

Figure 2 - A Muslim et al Rev1 Ref # 63422.docx
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Bentham Open - Sumaiya Azhar <sumaiya@benthamopen.com>
To: "a. muslim unsri" <a_muslim@unsri.ac.id>
Cc: editorial@benthamopen.org

Fri, Nov 23, 2018 at 2:45 PM

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Thank you very much for your kind efforts and providing us improved figures. Your provided figures have now been forwarded to our Technical team for checking, we will inform you soon about the quality of these figures.

We ensure you to provide the best quality services.

With best regards,

Sumaiya Azhar (Ms.)
Manager Graphics
Bentham OPEN
sumaiya@benthamopen.com

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

TOASJ :: Query Regarding Graphics Enhancement

3 messages

Bentham Open - Sumaiya Azhar <sumaiya@benthamopen.com>
To: a_muslim@unsri.ac.id
Cc: editorial@benthamopen.org

Mon, Nov 19, 2018 at 1:19 PM

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November 19, 2018

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With best regards,

S. Alavi

Manager Graphics

Bentham OPEN

editorial@benthamopen.org

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a. muslim unsri <a_muslim@unsri.ac.id> Tue, Nov 20, 2018 at 2:20 PM
To: sumaiya@benthamopen.com

Dear S. Alavi,

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Best regards,

A. Muslim

[Quoted text hidden]

Figure 2 - A Muslim et al Rev1 Ref # 63422.docx
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To: "a. muslim unsri" <a_muslim@unsri.ac.id>
Cc: editorial@benthamopen.org

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Bentham OPEN
sumaiya@benthamopen.com

[Quoted text hidden]

TOASJ :: Query Regarding Graphics Enhancement

2 messages

Bentham Open - Sumaiya Azhar <sumaiya@benthamopen.com> Mon, Nov 26, 2018 at 3:0
To: a_muslim@unsri.ac.id
Cc: editorial@benthamopen.org

Monday, November 26, 2018

Ref # 63422

Dear Dr. Muslim,

Thank you for your email in connection with your manuscript entitled **"A Rapid Bioassay t Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown an Root rot of Tomato "** submitted for publication in **"The Open Agriculture Journal "**. We ar pleased to inform you that the provided figure(s) being as per the publication standard wil be duly proceeded for publication.

We appreciate your kind cooperation in this respect.

With best regards,

Sumaiya Azhar (Ms.)
Manager Graphics
Bentham OPEN
sumaiya@benthamopen.com

From: a. muslim unsri [mailto:a_muslim@unsri.ac.id]
Sent: Tuesday, November 20, 2018 12:21 PM
To: sumaiya@benthamopen.com
Subject: Re: TOASJ :: Query Regarding Graphics Enhancement

Dear S. Alavi,

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Best regards,

A. Muslim

On Mon, Nov 19, 2018 at 1:20 PM Bentham Open - Sumaiya Azhar <sumaiya@benthamopen.com> wrote:

> # Query Regarding Graphics Enhancement
>
> November 19, 2018
>
> **Ref** # 63422
>
> Dear Dr. Muslim,

Thank you for submitting your manuscript entitled,**"A Rapid Bioassay to Evaluate Efficacy o Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato "** for possible publication in **"The Open Agriculture Journal "**. During graphics assessment, it has been observed that the figure(s) provided in your article have not been provided according to our requirements (which are given below for your convenience) and are not of the required quality, making the text and the graphics indistinct on reproduction or on attempting to adjust the figure(s) to the specified width.

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With best regards,

S. Alavi

Manager Graphics

Bentham OPEN

editorial@benthamopen.org

a. muslim unsri <a_muslim@unsri.ac.id> Tue, Nov 27, 2018 at 6:33 AM
To: sumaiya@benthamopen.com
Cc: editorial@benthamopen.org

Dear Dr. Sumaiya Azhar Manager Graphics,

Thank you very much for quick response regarding our revised figure 2.
We are realy appreciate that our revised figure 2 could be accepted and will be duly proceeded for publication.

thank you so much for your excellent cooperation

Best regard

A. Muslim
[Quoted text hidden]

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

Manuscript Acceptance letter | BMS-TOASJ-2018-43

11 messages

The Open Agriculture Journal Thu, Dec 6, 2018 at 12:12 PM
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To: a_muslim@unsri.ac.id
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Reference#: BMS-TOASJ-2018-43

Submission Title: A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato

Dear Dr. A Muslim,

I am pleased to inform you that your article entitled "A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato " has been accepted

for publication in "The Open Agriculture Journal" after independent peer review.

You will be pleased to know that Bentham Open has collaborated with Kudos to increase the portfolio of its services for Bentham authors. Kudos is among the preferred media for researchers. It is a web-based service that helps researchers maximize the visibility, usage of and citations to published articles (www.growkudos.com.) Kudos will be contacting you to register to use this service.

We have reached a decision regarding your submission to "The Open Agriculture Journal". The manuscript has been reviewed by the editorial board members of the journal and independent experts in the field. Based on the reviewers comments, I am delighted to inform you that the manuscript is now accepted for publication in the journal. On behalf of the Editorial Board, I would like to thank for your contribution and hope that you will consider this journal for future manuscripts.

We shall be most grateful if you could kindly agree to distribute the journal flyer at the next few conferences that you attend. Please download the flyer at https://benthamopen.com/journal-files/flyer/TOASJ-flyer.pdf

We wish to thank you for submission of the manuscript to

"The Open Agriculture Journal" and look forward to a continued collaboration in the
future.

Again, I sincerely thank you for submission of the manuscript in The Open Agriculture Journal.

Our decision is to: Accept Submission

With warm regards,

Ms. Sahar Iftekhar
Editorial Manager
E-mail: sahar@benthamopen.com
https://www.linkedin.com/company/benthamopen

Koji Kageyama <kageyama@green.gifu-u.ac.jp> Thu, Dec 6, 2018 at 1:24 PM
To: a_muslim@unsri.ac.id
Cc: kageyama@gifu-u.ac.jp, suwandi@fp.unsri.ac.id, rahmatpratamaunsri@gmail.com

Dear Muslim,

Congraturation for your paper's acceptance!!

It is so pleasure to hear it!

Koji

****

景山幸二

岐阜大学流域圏科学研究センター
〒501-1193　岐阜市柳戸１－１
Tel & Fax +8158293-2063

Koji Kageyama
Professor

River Basin Research Center
Gifu University
Gifu 501-1193, Japan
Tel & Fax +8158293-2063

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On 2018/12/06 14:12, The Open Agriculture Journal wrote:

> Reference#: BMS-TOASJ-2018-43
>
> Submission Title: A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato
>
> Dear Dr. A Muslim,

I am pleased to inform you that your article entitled "A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato " has been accepted for publication in "The Open Agriculture Journal" after independent peer review.

You will be pleased to know that Bentham Open has collaborated with Kudos to increase the portfolio of its services for Bentham authors. Kudos is among the preferred media for researchers. It is a web-based service that helps researchers maximize the visibility, usage of and citations to published articles (www.growkudos.com.) Kudos will be contacting you to register to use this service.

We have reached a decision regarding your submission to "The Open Agriculture Journal". The manuscript has been reviewed by the editorial board members of the journal and independent experts in the field. Based on the reviewers comments, I am delighted to inform you that the manuscript is now accepted for publication in the journal. On behalf of the Editorial Board, I would like to thank for your contribution and hope that you will consider this journal for future manuscripts.

*We shall be most grateful if you could kindly agree to distribute the journal flyer at the next few conferences

> that you attend. Please download the flyer at https://benthamopen.com/journal-files/flyer/TOASJ-flyer.pdf *
> [Quoted text hidden]

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a. muslim unsri <a_muslim@unsri.ac.id> Thu, Dec 6, 2018 at 4:40 PM
To: Koji Kageyama <kageyama@green.gifu-u.ac.jp>

Arigatou gozaimashita Kageyama Sensei...
Hope yuo will get success for everything..

Best Regard
A. Muslim
[Quoted text hidden]

---

a. muslim unsri <a_muslim@unsri.ac.id> Thu, Dec 6, 2018 at 4:53 PM
To: The Open Agriculture Journal <toasj@benthamopen.org>

Dear Ms. Dr. Sahar Iftekhar

Thank you very much for your good new emai.
It is a great honor for us, that our manuscript entitled: "A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato " have been accepted for publication in your Journal "The Open Agriculture Journal"

We will be very happy if our paper could be proceed soon and published in this year..
We will follow all the role and requirements in your Journal. Do not be hesitate to inform us regarding the role and the requirements.

Thank you very much

Best regard
A. Muslim
[Quoted text hidden]

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a. muslim unsri <a_muslim@unsri.ac.id> Mon, Mar 18, 2019 at 2:51 PM
To: The Open Agriculture Journal <toasj@benthamopen.org>

Dear Ms. Sahar Iftekhar

Reference#: BMS-TOASJ-2018-43

Submission Title: A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato

We are very pleased to hear that our article entitled "A Rapid Bioassay to Evaluate Efficacy of Hypovirulent Binucleate Rhizoctonia in Reducing Fusarium Crown and Root rot of Tomato " has been accepted for publication in "The Open Agriculture Journal" after independent peer review.

Since the information of accepted letter have been informed to us on December 5, 2018, We are very happy if you could inform us How is the process of our paper for publication in TOASJ.

We are realy hope that our Paper could be publised in TOASJ as soon as possible in a few days later.

Thank you very much for your kindness and excellent cooperation

Best Regard
A. Muslim
Sriwijaya University
Indonesia

On Wed, Dec 5, 2018 at 9:12 PM The Open Agriculture Journal <admin@bentham.manuscriptpoint.com> wrote:
[Quoted text hidden]

TOASJ Bentham Open <toasj@benthamopen.net> Tue, Mar 19, 2019 at 5:17 PM
To: "a. muslim unsri" <a_muslim@unsri.ac.id>
Cc: Qasit Malik <qasit@benthamopen.net>

Dear Dr. Muslim,

Thank you for your email. With reference to the below email, this is to inform you that we had sent you an email

regarding the proofs corrections of your article but did not receive any response. I have attached my email and composed version of your article for your convenience. I shall be grateful if you could kindly carefully check the manuscript for any potential errors, missing lines/paragraphs and errors in figures/diagrams etc.

Looking forward to your prompt response in this regard!

Note:
Please reply to this email at toasj@benthamopen.net otherwise your email will not reach me.

Regards,
Wajeeha Ahmed
Assistant Manager (Publication)
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2 attachments

Bentham Open Mail - BMS-TOASJ-2018-43.pdf
269K

TOASJ-18121201.pdf
683K

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a. muslim unsri <a_muslim@unsri.ac.id> Tue, Mar 19, 2019 at 8:13 PM

To: Suwandi fp <suwandi@fp.unsri.ac.id>, suwandi_unsri <suwandi_unsri@yahoo.com>, suwandi saleh <suwandi.saleh@gmail.com>

Ndi tolong dibantu ini email dari journal TOASJ...
Emailnyo sudah lamo ternyata tgl 19 Februari..

Makasih
A. Muslim
[Quoted text hidden]

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2 attachments

Bentham Open Mail - BMS-TOASJ-2018-43.pdf
269K

TOASJ-18121201.pdf
683K

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a. muslim unsri <a_muslim@unsri.ac.id> Tue, Mar 19, 2019 at 8:22 PM
To: TOASJ Bentham Open <toasj@benthamopen.net>

Dear Wajeeha Ahmed
Assistant Manager (Publication)

Thank you very much for your quick respond of our email.
I am really so sorry, I miss your email sent on February 18, so we did not reply your email at that time.

We are going to ceck in detail about the possible error of our manuscript and send it back soon.

Thank you very much for your kindness and excellent cooperation

Best Regard
A. Muslim

[Quoted text hidden]

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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Mar 20, 2019 at 9:33 AM
To: Suwandi fp <suwandi@fp.unsri.ac.id>, suwandi_unsri <suwandi_unsri@yahoo.com>, suwandi saleh <suwandi.saleh@gmail.com>

[Quoted text hidden]

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a. muslim unsri <a_muslim@unsri.ac.id> Wed, Mar 20, 2019 at 9:34 AM
To: Suwandi fp <suwandi@fp.unsri.ac.id>, suwandi_unsri <suwandi_unsri@yahoo.com>, suwandi saleh <suwandi.saleh@gmail.com>

[Quoted text hidden]

TOASJ Bentham Open <toasj@benthamopen.net> Wed, Mar 20, 2019 at 11:10 AM

To: "a. muslim unsri" <a_muslim@unsri.ac.id>
Cc: Qasit Malik <qasit@benthamopen.net>

Dear Dr. Muslim,

Thank you for your response. With reference to the below email, I request you to provide the corrections at your earliest so that manuscript can be proceeded further for publication without any further delay.

Looking forward to your prompt response in this regard!

Note:
Please reply to this email at toasj@benthamopen.net otherwise your email will not reach me.

Regards,
Wajeeha Ahmed
Assistant Manager (Publication)

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