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Dear Editor,

We would like to resubmit the revised manuscript entitled “Antifungal effect of magnolol and honokiol from *Magnolia officinalis* on *Alternaria alternata* causing tobacco brown spot” to your journal, “Molecules”. We sincerely thank for the reviewers comments, which make a great improvement for our manuscript. We were also very pleased to see that all reviewers recognized the novelty and potential significance of our work. We have added some new data supposed by reviewers, described in detail below, and revised the manuscript to address reviewers’ comments. Here are our point-by-point responses:

Response to Reviewer 1 Comments

Point 1:

I think that authors had improved their manuscript according the reviewer ’s suggestion. All my comments had been answered, but the manuscript still need some correction before it should be considered for publication in your Journal and the language should be improved and corrected by native speaker.

Response:

Considering the Reviewer’s suggestion, the manuscript has been corrected and improved by native speaker.

Point 2:

Line 105 I don't understand the terminus mycelial radial growth inhibition rate – I think the sentence should be changed „The growth inhibition of *A.alternata* was 77% in the presence of 0,1mg/ml of magnolol resp. 91% in the presence of honokiol.

Response:

We have revised this part according to the Reviewer's suggestion.

The growth inhibition of *A. alternata* was 77% and 91% in the presence of 0.1 mg/ml of magnolol and honokiol, respectively.

Point 3:

Line 107 authors had written: And the antifungal activity was much better than 0.1 mg/mL eugenol (11%) and ? 0.001 mg/mL carbendazim (58%)?.I cannot agree with such a claim because carbendazole is more effective than magnolol resp. honokiol. It is shown in Tab.1 that at concentration of 0.001mg /mL carbendazol was able to inhibit the growth of *A. alternata* on 58% and the obtained inhibition of test compounds was only 7% respectively 9%. “

Response:

Considering the Reviewer's suggestion, we have corrected as follow:

The antifungal activity of carbendazim was much higher than that of magnolol and honokiol (Table 1), when used at concentration with 0.001 mg/mL.

Moreover, the antifungal activity was much higher than eugenol (0.1 mg/mL), which showed 11% inhibition of mycelial growth. On the basis of the present results which are also in agreement with the reported by Edris and Farrag (2003), eugenol was completely inactive on *Sclerotinia sclerotiorum* (Lib), *Rhizopus stolonifer* (Ehrenb. exFr) Vuill and *Mucor* sp. (Fisher), but mixing eugenol and linalool in a ratio similar to their concentrations in the original oil was found to enhance the antifungal properties oil indicating a synergistic effect[29].

Point 4:

Line 107 authors had written: Could be the effectivity of compounds compared in concentration expressed as mg/mL. „Would it not be better to compare the activity of compounds when their concentrations are expressed in mol / L ?“

Response:

Thanks for the precious comment and suggestion proposed by the reviewer 1. We understand that it is better to compare the activity of compounds when their

concentrations are expressed in mol / L, this may better reveal the possible antifungal activity of magnolol and honokiol. However, according to the specification of carbendazim, it was expressed as mg/mL.

Point 5:

Line 115 Chapter 2.3. So was there any synergic effect of these two compounds or not ?

Response:

We are very sorry for our poor writing. We have corrected “The results showed that there was not any synergic effect of these two compounds.”

Point 6:

Authors should – improve English line 116-123

This sentences are really hard to understand.: line 119-121 The results showed that the mycelial growth was better than other 6 volume ratio, with a radial growth inhibition of 91%, under the condation of the volume ratio of magnolol and honokiol(0:1),. When the volume ratio of magnolol and honokiol was 1:4, 89% of mycelial growth was inhibited. When the volume ratio was 1:0, the growth inhibition was only 77%.

Table 2. I don ´t understand the term concentration ratio of magnolol(0.1 mg/mL) and honokiol (0.1 mg/mL) For example the ratio 1:4; what is the concentration of magnolol a what is the concentration of honokiol? Should it not be the volume ratio 1:4?

Response:

Considering the Reviewer’s suggestion, we have corrected as follow:

In order to examine the synergistic effect of a combination of two compounds on the mycelial growth of *A. alternata*, we combined two compounds in different ratios and tested their effect *in vitro* assays (Table 2). The results showed that the mycelial growth inhibition was higher than other six volume ratios, with radial growth inhibition of 91%, under the condition of the volume ratio of magnolol and honokiol (0:1). When the volume ratio of magnolol and honokiol was 1:4, a growth inhibition of 89% of mycelial growth was inhibited. When the volume ratio was 1:0, the growth inhibition was only 77%. The results showed that there was not any synergic effect of these two compounds.

Point 7:

Table 2. I don ´t understand the term concentration ratio of magnolol(0.1 mg/mL)

and honokiol (0.1 mg/mL) For example the ratio 1:4; what is the concentration of magnolol a what is the concentration of honokiol? Should it not be the volume ratio 1:4?

Response:

We are very sorry for our poor English. Synergistic effect of magnolol and honokiol was tested against *A. alternata*. magnolol and honokiol (0.1 mg/mL each) were mixed at volume ratio of 1:0, 1:1, 1:4, 1:9, 9:1, 4:1 and 0:1 in a total volume of 5 mL, and added to sterilized Petri dishes (90 mm diameter) containing PDA medium (15 mL).

Point 8:

Line 126 Change the title 2.4. The Inhibition Effects of Magnolol and Honokiol on Morphology and Mycelial of *A. alternata*. Inhibition effect on morphology? I recommend : Growth inhibition and the effect on morphology...

Response:

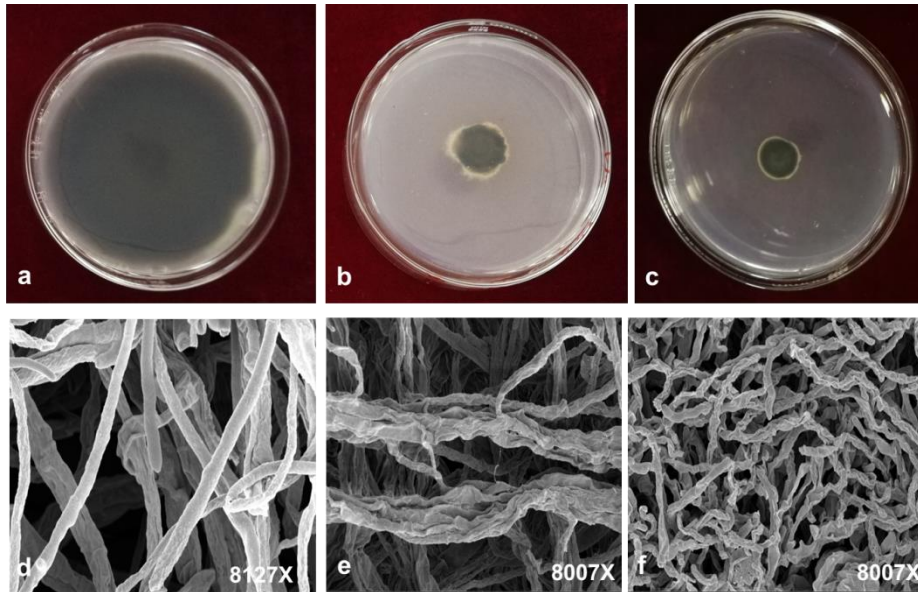
We have made correction according to the Reviewer's comments.

Point 9:

Figure 1 Indication of magnification is missing

Response:

We are very sorry for our incorrect writing, and indication of magnification has been added.



Point 10:

Lines 151 -152 Is the conductivity unit correct $\mu\text{S}/\text{cm}$ u- stays for micro? Should it be not indicated with the Symbol „ μ “ and should not the “s “ not written with capital „S“ for Siemens

Please write *in vitro* in italic fond, in whole text

Response:

We are very sorry for our incorrect writing, and the conductivity unit has been corrected “ $\mu\text{S}/\text{cm}$ ”, and in whole text it was writed *in vitro* in italic fond.

Point 11:

177 change alternate to alternate

Response:

We are very sorry for our incorrect writing, and alternate has been corrected alternata.

Point 12:

187 two comma

Response:

We are very sorry for our incorrect writing, and one comma has been deleted.

Point 13:

Explain the paragrapgh line 257-258 „Magnolol and honokiol (5 mL) were added to sterilized Petri dishes (90 mm diameter) to the concentrations of 0.001, 0.005, 0.01,

0.10, 1.00, 3.00, 5.00, 7.00 mg/mL, ?

and 5 mL meugenol (0.1g/mL) and carbendazim (0.001g/mL) mixed with PDA medium (15 mL). „

Were added to concentration??? And mixed with 15mL PDA??? I am sorry but I really don't understand and have more questions "What was the real final concentration of magnolol or honokil in the growth medium? Do I understand it correct? For example if 5mL of magnolol (7mg/mL) was dilluted with 15mL of PDA?It was diluted 4 times so the concentration in the growth medium is not 7mg/mL but 4 times lower? Is my consideration correct or not?

Response:

We are very sorry for our poor English. The reviewer consideration is correct. For example if 5mL of magnolol (7mg/mL) was dilluted with 15mL of PDA, It was diluted 4 times so the concentration in the growth medium is not 7mg/mL but 4 times lower. We has been corrected as follow:

Magnolol and honokiol (5 mL) were added to sterilized Petri dishes (90 mm diameter) to the concentrations of 0.001 mg/mL, 0.005 mg/mL, 0.01 mg/mL, 0.10 mg/mL, 1.00 mg/mL, 3.00 mg/mL, 5.00 mg/mL, 7.00 mg/mL, and 5 mL eugenol (0.1 g/mL) and carbendazim (0.001 g/mL) mixed with PDA medium (15 mL).

Point 14:

"Line 257 change meugenol to eugenol"

Response:

We are very sorry for our incorrect writing, and meugenol has been corrected eugenol.

Point 15:

"Line 260 and answer for comment 13 of Reviewer 1. Why was diluted Tween20 used for control experiment instead the solvent DMSO? When authors had used 5ml of tested compounds dissolved in DMSO and 15ml of PDA was added, there was high content of DMSO in the growth medium. It is known that DMSO inhibits the growth of fungi (max 2% of DMSO could be used without growth inhibition of fungal cells) moreover DMSO is causing membrane damage."

Response:

We are very sorry for our incorrect writing. We searched scientific literature and knowed moreover DMSO is causing membrane damage. Magnolol and honokiol were dissolved in DMSO (1000 mg/mL), and diluted to the required concentration with Tween-20 and distilled water (1:1000 v/v).Forexample, 5 mL honokiol (5 mL/mL) were added to sterilized Petri dishes (90 mm diameter) mixed with PDA medium (15 mL). In the 20 ml solution, DMSO content just was 0.1%, it can be ignored. So diluted Tween20 was used for control experiment instead the solvent DMSO.

Point 16:

“Line262 After incubation for 7 days at $25 \pm 2^{\circ}\text{C}$ for 7 days, the colony diameter measured. "was “ is missing in the sentence.”

Response:

We are very sorry for our incorrect writing, and was has been added.

Point 17:

“Chapter.4.4; 4.9 Synergism; Antifungal test. I see the same problem with DMSO as in 4.3”

Response:

We are very sorry for our incorrect writing. We searched scientific literature and knowed moreover DMSO is causing membrane damage. Magnolol and honokiol were dissolved in DMSO (1000 mg/mL), and diluted to the required concentration with Tween-20 and distilled water (1:1000 v/v).Forexample, 5 mL honokiol (5 mL/mL) were added to sterilized Petri dishes (90 mm diameter) mixed with PDA medium (15 mL). In the 20 ml solution, DMSO content just was 0.1%, it can be ignored. So diluted Tween20 was used for control experiment instead the solvent DMSO.

Point 18:

““Chaper 4.3 and 4.9 could be merged.”

Response:

According to the Reviewer’s suggestion, the alkaline water in the revised draft is as follow:

3.3. Isolation and purification of active compounds and structure analysis

The stem bark of *M. officinalis*, was rolled up into a column, brown and spicy (Figure 6). With the method of alkali-extraction and acid-precipitation, 100 g dried bark was ground into powder by disintegrator XL600B (Xiaobao Corporation, China), and extracted three times with 90% Methanol. The resulted crude extract (1500 mL) was dried to a paste and then incubated with two times quicklime for 24 h. After washing three times using five times diluted hydrochloric acid, the extract paste was concentrated. After the concentrating step, the extract was dissolved in chloroform and isolated with three volumes of alkaline water (0.5% NaOH solution). The crystals were filtered out from the solution by adjusting for pH value to 7. After drying at a low temperature, the solution that was filtered from the crystal with petroleum for 1h was naturally cooled to room temperature, and the final product was analyzed by a liquid chromatography-mass spectrometry (LC-MS). The structures were identified by ^1H and ^{13}C NMR spectra. ^1H and ^{13}C NMR spectra were obtained with Bruker Avance III 500 MHz (Bruker Corporation, Karlsruhe, Germany). HPMS were recorded on a Triple TOF 5600+ system (AB SCIEX, USA). The high-performance liquid chromatography was performed on an Agilent 1260 liquid chromatography system equipped with a Zorbax SB-C18 column (particle size 5 μm , dimension 150 mm $\times$ i.d. 9.4 mm, flow rate 7 mL min $^{-1}$, Agilent Technologies) .

Thank you for your consideration of our manuscript.

Yours sincerely,

Yahan Chen, Ph.D.