SDI FINAL EVALUATION FORM 1.1

PART 1:

Journal Name:	International Research Journal of Pure and Applied Chemistry
Manuscript Number:	**Ms_IRJPAC_33353**
Title of the Manuscript:	**Detection of Phytotoxin Produced from Leaf, Neck and Finger Blast Disease Causing Magnaporthe grisea through GC-MS Analysis**
Type of Article:	**Original Research Article**

PART 2:

FINAL EVALUATOR'S comments on revised paper (if any)	**Authors' response to final evaluator's comments**
I appreciate the interest of the study, but the authors did not reply to all the comments of my first evaluation, and in my opinion they should revise some essential parts of the manuscript. **They did not edit the manuscript, as they only introduce a couple of points concerning Rf values. The manuscript is largely identical to the first version. I will try to be precise with the minimum changes to be introduced** a) **Abstract:** What do you mean with the word "discriminated"? There is no discrimination. Delete this word unless any attempt of discrimination will be introduced in the paper discussion. b) **Lines 85-92** "The aqueous fraction after solvent separation containing toxic activity was applied to a Sephadex G-75 (Sigma, USA) superfine column (2.5x2.5 cm, Pharmacia, USA) and eluted with double distilled water at room temperature. Fractions (5 mL) were collected at a flow rate of 5 mL min^{-1} using Bio-Rad automated econosystem (Biorad, USA) and the column elute was monitored by UV monitor and recorder of the chromatography unit based on the absorbance at 280 nm. The peak fractions were combined and evaporated to dryness *in vacuo* at 40°C, dissolved in 5 mL of distilled water to get a clear homogeneous syrup, freeze dried and stored at -20°C. The partially purified toxin was used to analyze the biological functions of the toxin in all further studies". ***Questions to be cleared up: Is really the column 2.5 cm long? Are fractions of 5 ml? How many large fractions did you get from the small column? Column dimensions and fraction volume do not reconcile. Are the peak fractions combined?. Why did you perform a gel-filtration chromatography and finally the isolated peaks are combined?*** c) **Lines 107-115. "**Spots were visualized by spraying with various spraying reagents to find different compounds present in the extract. Compounds were detected by spraying with 1% ~~per cent~~ Ferric chloride reagent for flavonoids, Dragendeoff's reagent for alkaloids, Liebermann-Burchard reagent for steroids and Anisaldehyde-sulphuric acid for sugars. Presence of compound was indicated by specific colour spots. All the spots were observed under UV light (254 nm). The relation to front (R*f*) of the spots developed on the TLC plate were recorded using the formula given below. $$Rf \text{ value} = \frac{\text{Distance (cm) moved by the solute from the origin}}{\text{Distance (cm) moved by the solvent from the origin}}$$" ***My comments concerning this part:*** ***1) The calculation of Rf values in TLC s well known. Delete the formulae. It is unneeded.*** ***2) Visualization with all spraying agents is all right, but they are not shown. The only TLC shown in results is visualized under UV and iodine test, but this test is not described in this section. Sorry, this is very difficult to understand. It should be a correlation among methods of TLC detection and***	**Please see my revised manuscript for your kind consideration.**

results. Methods describe a series of test that are never used. Opposite, the visualization test shown in results (Plate 1) is not described in method.

3) ***In the reply letter, authors state that toxin is a protein. This should be included in the paper. Are the three bands different forms of the same protein?***

d) ***Line 145-146.*** **"**Compounds were identified by absorbance at nm over 10 to 25 min (total analysis time 35 min)". ***As I said before, This is meaningless. Absorbance at nm? How many nm? The same nm for all compounds?***

e) ***Concerning my general comments in the first review: "The identification of the proposed phytotoxins in this paper is not totally demonstrated. The list of compounds listed in GC_MS is too large and speculative. There is no warranty that they are phytotoxins. Identification of some of them is also doubtful. There is no direct relationship between the TLC and GC_MS. There is not MS data. There are too many minor errors and ambiguities throughout the text. Reagents listed in M&M are not used in results, and opposite (i.e iodine tank). Rf values are relative values, and they are useless in the abstract. Manuscript should be simplified, and only well identified compounds with chance to be phytotoxins would be described in the discussion. Materials and Methods, Results and Discussion should be consistent. Conclusion is too general."***

Authors reply***:*** **"**Yes agreed with this, It is only profiling of partially purified compounds",
…. but they do nothing. Discussion and conclusion are identical. At least, they should state some relationship between toxin detected by TLC and toxins detected by GC_MS. Do authors propose that the TLC toxin is a protein? If so, they should say it. The word protein does not appear throughout the paper. Is the identification of all compounds detected and identified by GC_MS a new proposal? Is there any discrimination? These points should be discussed to improve the paper. Discussion is not a discussion, it is a list of putative compounds identified in the three parts (leaf, neck and finger).