# Modification of Sporulation Medium for Isolation and Identification of *Colletotrichum Gloeosporioiedes* - A Causal Agent of Anthracnose of *Mangifera Indica* L

Ashraful Alam[1,2,*], Sanjoy Kumar Adhikary[2] and Mahtalat Ahmed[2]

[1]*Department of Plant Pathology, Faculty of Agriculture, Patuakhali Science and Technology University, Patuakhali - 8602, Bangladesh*

[2]*Agrotechnology Discipline, Khulna University, Khulna-9208, Bangladesh*

**Abstracts:** In the present study, different isolates of *Colletotrichum gloeosporioiedes* were collected from different mango growing areas of Bangladesh. Pathogenecity was proved by using healthy excised leaves in laboratory conditions. Effects of mycelial growth and sporulation (formation of acervuli) on six different culture media were tested *in vitro*. The results revealed significant differences ($p<0.05$) mycelia growth among the media. The results also indicated that the mycelial growth was maximum in Potato Dextrose Agar (12.8mm) media and the lowest in Green Bean Agar (8.0mm). The study disclosed significant difference ($p<0.05$) of acervuli formation among the media. Among the 6 different media the maximum acervuli were produced in HLEA (4.0 per square. cm) medium. But the sporulating characters of the fungus were not produced well in PDA and HLEA media rather than OMA. Thus, to find out the best composition of OMA for further research was conducted to obtain better growth and acervuli production and sporulating characters. The results revealed significant difference ($p<0.05$) of different characters among the concentration of OMA, and 20g/L OMA produced significantly maximum setae, conidia and sticky masses of conidia than others. The study suggested that Oat Meal Agar (20g/L) composition was the best medium for the sporulation of *C. gloeosporioiedes* (Setae, conidia and sticky masses of spores).

**Keywords:** Acervuli, *Colletotrichum gloeosporioiedes*, media, mycelial growth, sporulation.

## 1. INTRODUCTION

Mango (*Mangifera indica* L.) is a juicy stone fruit of Anacardiaceae family, a highly valued fruit crop in Bangladesh [1]**.** But the production is challenging due to a collection of opportunistic pathogens [2]. Among the pathogens (*Colletotrichum gloeosporioiedes),* causes an anthracnose disease which is the most important limiting factors for production due to heavily losses [3]. In Bangladesh 25 to 30% losses of total production of mango are due to anthracnose and stem end rot diseases [4]. In Bangladesh, anthracnose is the most familiar disease among all of the diseases of mango, [5]. Anthracnose is a common disease of mango in all mango producing areas of the world, caused by *C. gloeosporioiedes* [2, 6-12]. It is the major postharvest disease of mango. The disease occurs as quiescent infections on immature fruit and the damage inside mango, which is the most serious disease affecting the fruit at the ripening stage as well as postharvest period [13] leading rotten and poor quality of fruits, which has encouraged laboratory and clinical research. This fungal pathogen produces severe lesions on young mango leaves, while lesion is most commonly observed on mature mango leaves. Many other *Colletotrichum* species have a selective sporulating media but unfortunately, there is no sporulating media of *C. gloeosporioiedes* [14]. The priority of this study is to select the best sporulating as well as mycelial growth medium for identifying morphologically with the lowest use of food materials because of quick sporulation. Observations on exhibited variation of the different morphological characters *viz*. color, setae, acervuli and conidia / spore are also important. The selection of an efficient medium is leaded to improve disease management practices [2]. Accurate and rapid identification of pathogens is necessary for appropriate management of plant diseases [13]. Therefore, the present study was undertaken to identify pathogenic fungus causing anthracnose of mango and to select optimal media composition for mycelial growth and quick sporulation. The goal of this study was to select an efficient medium for the better growth and development (sporulating) of *C. gloeosporioiedes* which will be contributed to the future research.

## 2. MATERIALS AND METHODS

### 2.1. Isolation and Identification of the Pathogen

The pathogen was isolated by tissue planting method on potato dextrose agar (PDA) [15]. Mango leaves and fruits showing characteristics symptom were collected from the mango growing areas of

*Address correspondence to this author at the Department of Plant Pathology, Faculty of Agriculture, Patuakhali Science and Technology University, Patuakhali - 8602, Bangladesh;
Tel: +8801717285752; Fax: +88-04427-56009; E-mail: ashraful7@gmail.com

**E-ISSN: 2311-858X/16** 

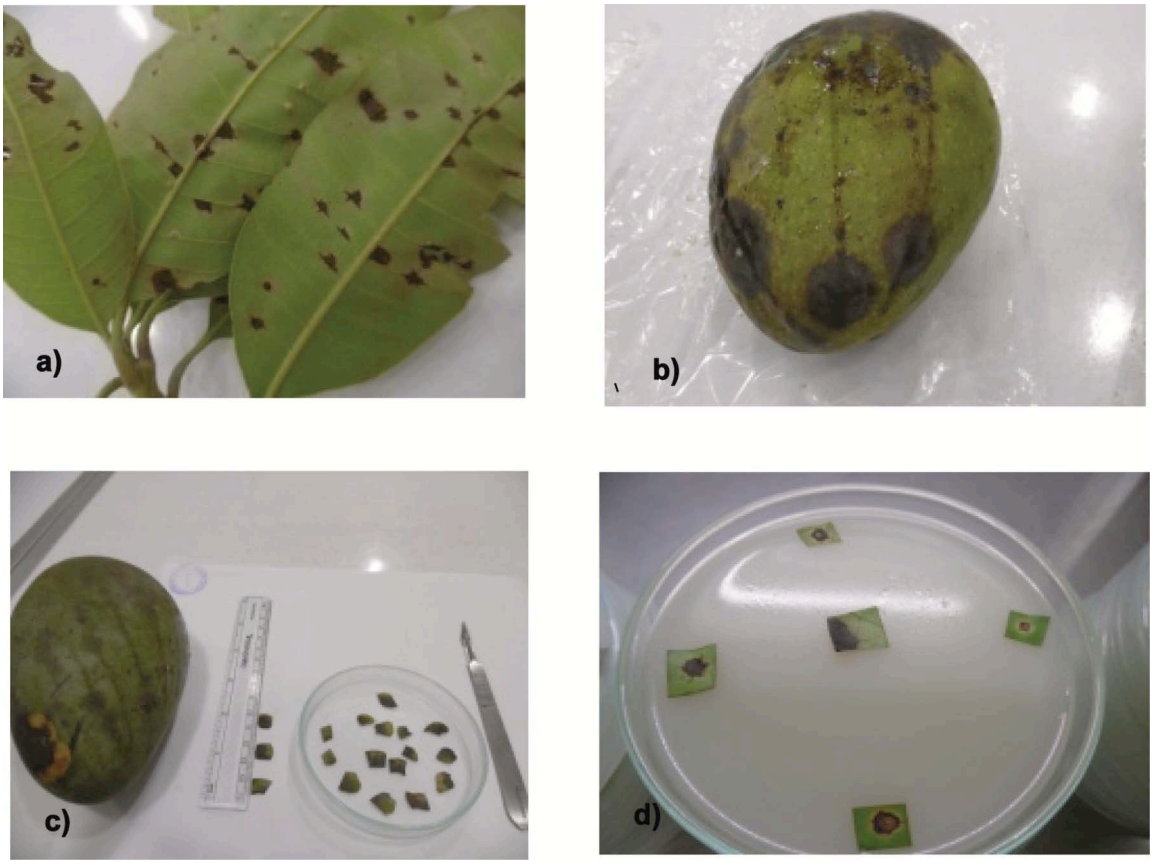


**Figure 1:** Collection and preparation of samples for placement on different culture media: (**a**) infected mango leaves, (**b**) anthracnose on mango, (**c**) 5mm cut sample, and (**d**) placing the prepared sample on media.

Satkhira, (January' 2015) the southern region of Bangladesh (Figure **1a**, and **1b**). The infected leaves and fruits were cut into 5 × 5mm [16]. Surface sterilized with 1% Sodium hypochlorite (NaOCl) for 1min. [17] and washed by sterile water and dried with sterilized filter paper (What man qualitative filter paper, grade 1). Then the prepared samples were placed on PDA containing Petri-plates (Figure **1d**). The plates were incubated at room temperature (25$^{o}$C) and observed for the fungal growth [18]. The pathogen was recognized up to species level depending upon their cultural and morphological characters [19]. Prepared the glass slides were placed under the compound microscope and observed images for the presence of conidia with ten times (10x) and hundred times (100x) magnifications.

### 2.2. Pathogenecity Test

The identified isolate of *C. gloeosporioiedes* (Figure **3a**) was confirmed by Koch's Postulate methods [20]. Healthy excised mango leaves, 8cm in middle portion were placed in Petri-plates with 5 replications. The conidial suspension 5 × $10^{6}$ spore/ml (supplemented with 0.01% Tween 80 in a ratio of 1:1 v/v) was prepared [21]. This suspension was tested by the Drop Inoculation Method on the excised mango leaves. The leaves were slightly injured by Pin Prick Method [22] and then inoculation by dropper (pre sterilized by 70% ethanol) [23]. The excised leaf inoculated with only sterile distilled water served as control. The fungus artificially inoculated in leaves showing the symptom was re-isolated and compared with the original pathogen.

### 2.3. Preparation of Culture Media and Design of the Experiment

We used six culture media to identify the most appropriate one for the mycelial growth of the fungus. Each medium was prepared with 20g agar in 1L of distilled water, at which pH was adjusted 6.5 with 15 psi for 20 mins at 121$^{o}$C temperature. The culture media were 1) PDA: Potato Dextrose Agar (200g peeled potato slices, 20g dextrose) [24], 2) OMA: Oat Meal Agar (60g oat meal) [25], 3) CMA: Corn Meal Agar (40g corn meal) [26], 4) GBA: Green Bean Agar (400g green bean) [27], 5) MPA: Mango Pulp paste Agar (20g pulp) [28], 6) HLEA: Host Leaf Extract Agar (300g leaves) [28] (Figure **2a**). In each Petri dish (9cm in diameter**)** [29] 20ml media was poured to maintain uniformity. The experiment was conducted with

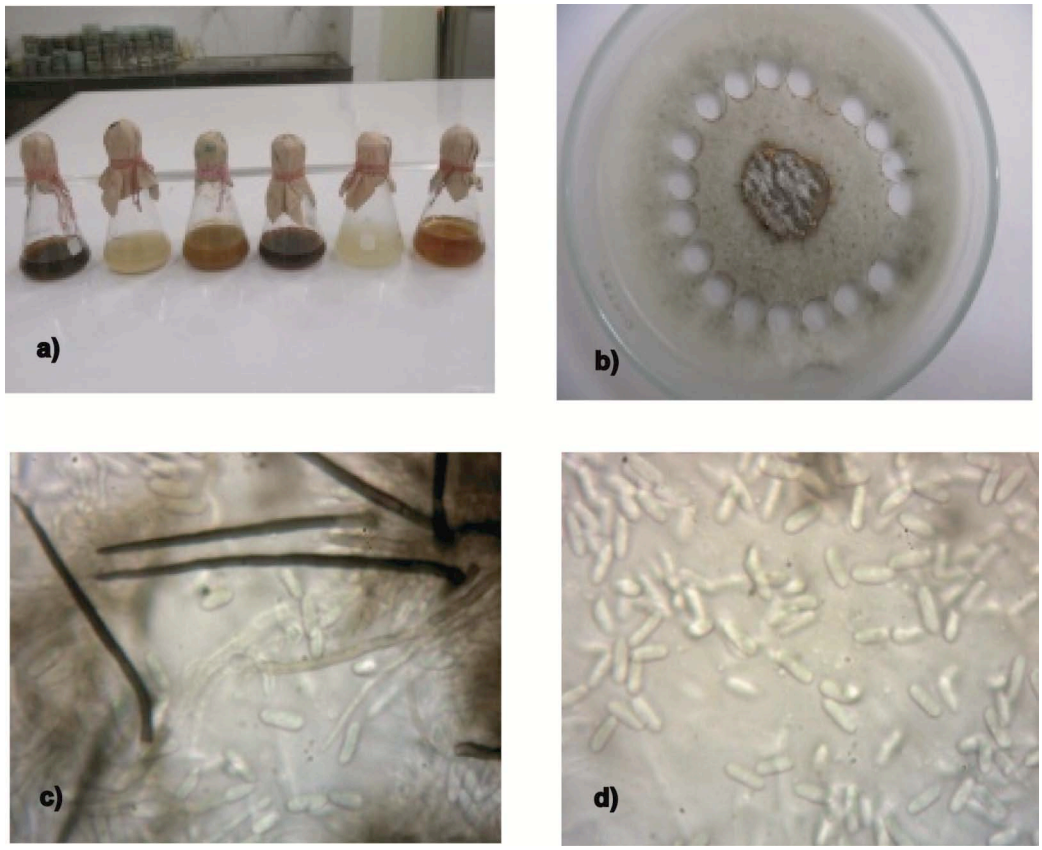


**Figure 2:** Preparation of different media for mycelial growth and identification of *C. gloeosporioiedes* with setae and conidia: (**a**) media preparation, (**b**) 5mm mycelia block cut, (**c**) setae, and (**d**) conidia.

completely randomized design (CRD) with five (5) replications. Seven days old cultures were cut into 5mm with the help of a block cutter (Figure **2b**) and placed them oppositely (upper surface turns into bottom surface) in the center of the plates. Colony diameter was measured daily after inoculation until the mycelia touches the edge of Petri dishes. The acervuli were counted from the first acervulus observed till the whole plate covered. Average Linear Growth Rate (ALGR) and Average acervuli were counted and measured by the formula Jahan, *et al*., 2013 [13]. The different morphological characters viz. color visually, presence of acervuli, masses of spores under stereo microscope, setae presence or absence and shape, colored or hyaline, uni or multi-cellular conidia were recorded under compound microscope with 10x, 100x magnifications [30] (Figure **2**).

### 2.4. Evaluation of Sporulation on Different OMA Media

Five different compositional Oat Meal Agar (OMA) media (50g, 40g, 30g, 20g, and 10g oat meal/L) were used to select the most suitable one for the sporulation of the *C. gloeosporioiedes*. Each medium was prepared with 20g agar in 1L of distilled water, at which pH was adjusted 6.5 with 15 psi for 20 mins at 121$^{o}$C temperature [18]. In each Petri dish 20ml media was poured with five (5) replications. The acervuli were counted from the first acervulus observed to till the whole plate covered by stereo microscope.

### 2.5. Statistical Analysis

The statistical data analysis was carried out by using Microsoft Excel 2007, and Statistica software (Version 10). ANOVA (One way analysis of variance) was carried out to recognize the difference of mycelia growth among different media. ANOVA was also conducted to find out the significant difference of different concentration of OMA media on mycelia growth. ANOVA was also carried out to observe the significant difference of mean acervuli per square cm among different culture media. One way analysis of variance was conducted to identify the significant difference of mean acervuli per square cm among the concentration of OMA media. Tukey's Post Hoc tests were carried out for comparison of means of mycelia growth (mm/day) among the culture media. Tukey's Post Hoc tests were also carried out for comparison of means of mycelia growth (mm/day) among different concentration of OMA media.

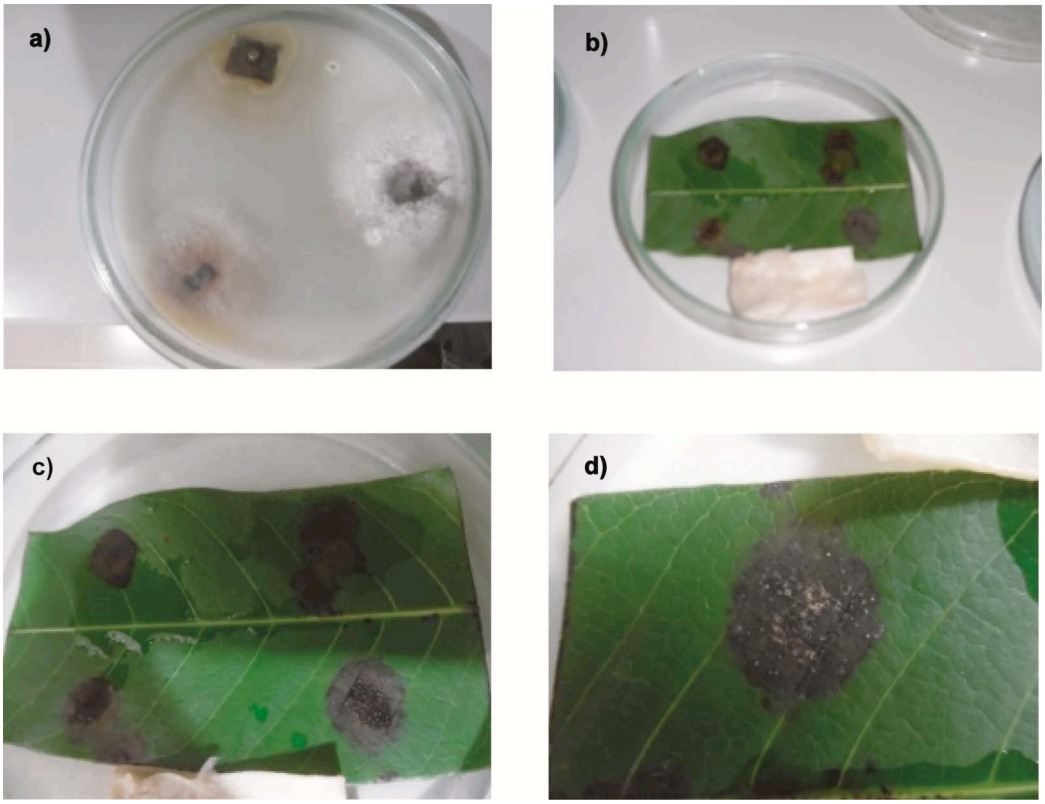


**Figure 3:** Pathogen development and isolation for inoculation on healthy mango leaves: (**a**) isolation of the pathogen, (**b**) fungus inoculation on healthy mango leaves after 8 days, (**c**) fungus inoculation on healthy mango leaves after 10 days, and (**d**) fungus inoculation on healthy mango leaves after 12 days.

## 3. RESULTS

### 3.1. Isolation and Identification of the Pathogen

A single organism was associated with the symptoms of anthracnose on the collected mango leaves. The isolates were identified based on morphological characters. We identified the presence of unicellular, hyaline and oval shape conidia (Figure **2c**) and needle like black color setae (Figure **2d**) as *C. gloeosporioiedes*.

### 3.2. Pathogenecity Test

We determined that the collected isolate were able to cause disease on excised mango leaves in Petri dishes under laboratory conditions after inoculation from pathogenecity test. Symptom appeared after eight days of inoculation (Figure **3b**). Small brown spot on leaves and the spots gradually enlarged after ten days (Figure **3c**), centre of the lesions turned dark brown with numerous acervuli with masses of spores was observed after twelve days (Figure **3d**) under stereo microscope. The glass slide was prepared and

**Table 1: Effect of Mycelial Growth on Different Media for Six Continuous Days**

| Media | Mean Mycelial Growth (mm/day) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Average |
| PDA | 12.2a | 10.5a | 13.1a | 13.5a | 14.1a | 13.5a | 12.8a |
| OMA | 08.3c | 08.2b | 09.7b | 09.7c | 10.4b | 10.6b | 9.5bcd |
| CMA | 08.1c | 07.9b | 08.6c | 08.7d | 09.7b | 09.5c | 8.7cd |
| GBA | 04.6d | 05.6c | 08.6c | 09.1cd | 10.1b | 9.9bc | 8.0bc |
| MPA | 09.7b | 11.4a | 12.9a | 12.7b | 13.5a | 13.6a | 12.3a |
| HLEA | 08.1c | 08.2c | 09.6b | 09.6c | 10.2b | 10.1b | 9.3d |

**Note:** PDA - Potato Dextrose Agar, OMA - Oat Meal Agar, CMA - Corn Meal Agar, GBA - Green Bean Agar, MPA - Mango Pulp paste Agar, and HLEA - Host Leaf Extract Agar. The same letter(s) in column indicate(s) no significant difference at $p<0.05$.

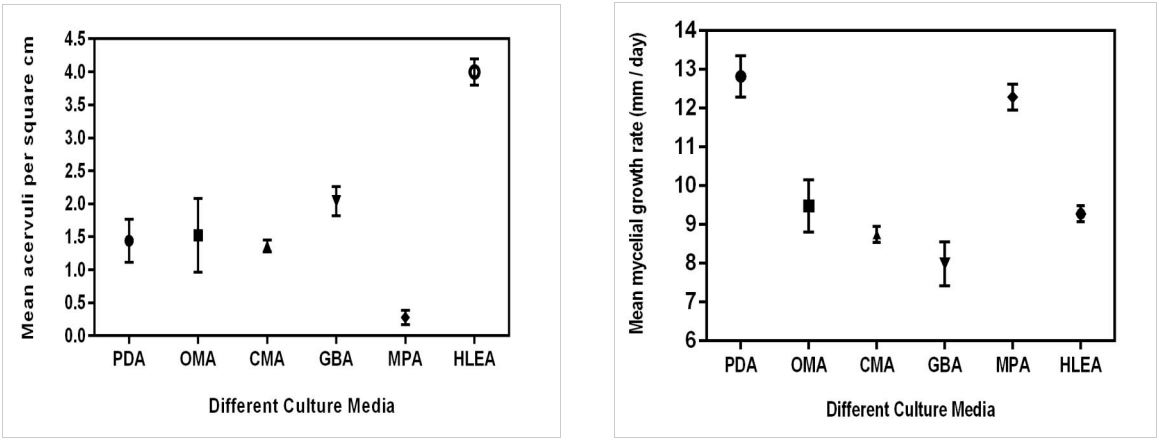


**Figure 4:** Formation of acervulus on different media per square cm. Note: PDA **-** Potato Dextrose Agar, OMA - Oat Meal Agar, CMA - Corn Meal Agar and GBA - Green Bean Agar, MPA - Mango Pulp paste Agar, and HLEA - Host Leaf Extract Agar.

observed under compound microscope 10x and 100x magnifications. The presence of conidia was also similar to the original isolate.

### 3.3. Effect of Culture Media

All the 6 media exhibited mycelial growth of *C. gloeosporioiedes*. The highest mean colony diameter was recorded in Potato Dextrose Agar (PDA) (12.8mm) and the lowest in Green Bean Agar (GBA) (8.0mm) followed by MPA (12.3mm), OMA (9.5mm), HLEA (9.3mm), CMA (8.7mm) (Table **1**). The results revealed significant differences ($p<0.05$) of mean mycelia growth (mm/day) among the culture media. Tukey's Post Hoc tests were also revealed the comparison of means of mycelia growth (mm/day) among the culture media (Table **1**). Maximum mean acervuli were counted in HLEA (4.0 per square cm) and the lowest in MPA (0.28 per square cm) followed by GBA (2.16 per square cm), OMA (1.52 per square cm), PDA (1.44 per square cm), CMA (1.36 per square cm) (Figure **4**). The results

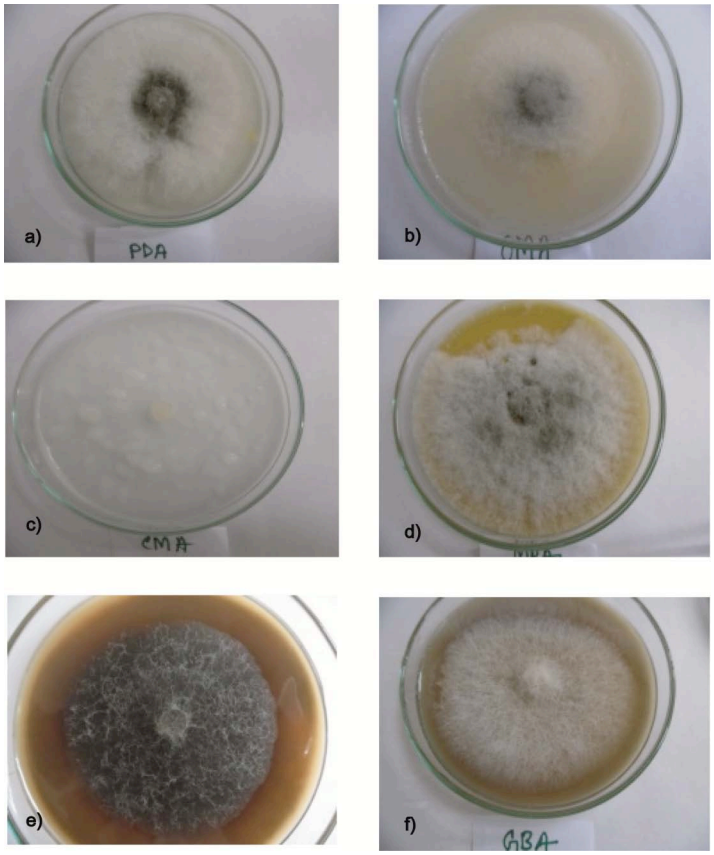


**Figure 5:** Cultural view of different media: (**a**) PDA **-** Potato Dextrose Agar, (**b**) OMA - Oat Meal Agar, (**c**) CMA - Corn Meal Agar, (**d**) GBA - Green Bean Agar, (**e**) MPA - Mango Pulp paste Agar, and (**f**) HLEA - Host Leaf Extract Agar.

**Table 2: Cultural Characters in Different Media During the Experiment**

| Media | Different Morphological Characters | | | |
|---|---|---|---|---|
| | Color | Setae | Acervuli | Conidia/Spore |
| PDA | White | Nil | Present | Present |
| OMA | White | Present | Present | Present |
| CMA | White thin | Present | Present | Present |
| GBA | White fluffy | Nil | Present | Present |
| MPA | Grayish white | Nil | Nil | Nil |
| HLEA | Brownish light | Nil | Present | Present |

**Note:** PDA - Potato Dextrose Agar, OMA - Oat Meal Agar, CMA - Corn Meal Agar, GBA - Green Bean Agar, MPA - Mango Pulp paste Agar, and HLEA - Host Leaf Extract Agar.

revealed significant difference ($p<0.05$) of mean acervuli per square cm among the culture media (result not shown). The fluffy growth was found in GBA and thin growth in CMA (Figure **5**). The setae and conidia were recorded in OMA and CMA medium followed by other 4 media with stereo microscopic observations (Table **2**).

### 3.4. Mycelial Growth and Counting the Acervuli on OMA Media

Mycelial growth of *C. gloeosporioiedes* was tested among the five OMA media (Figure **6**). The highest mean colony diameter was recorded in 20g OMA/L (10.2mm) and the lowest in 40g OMA/L (9.3mm) followed by 10g OMA/L (9.9mm), 50g OMA/L (9.6mm),

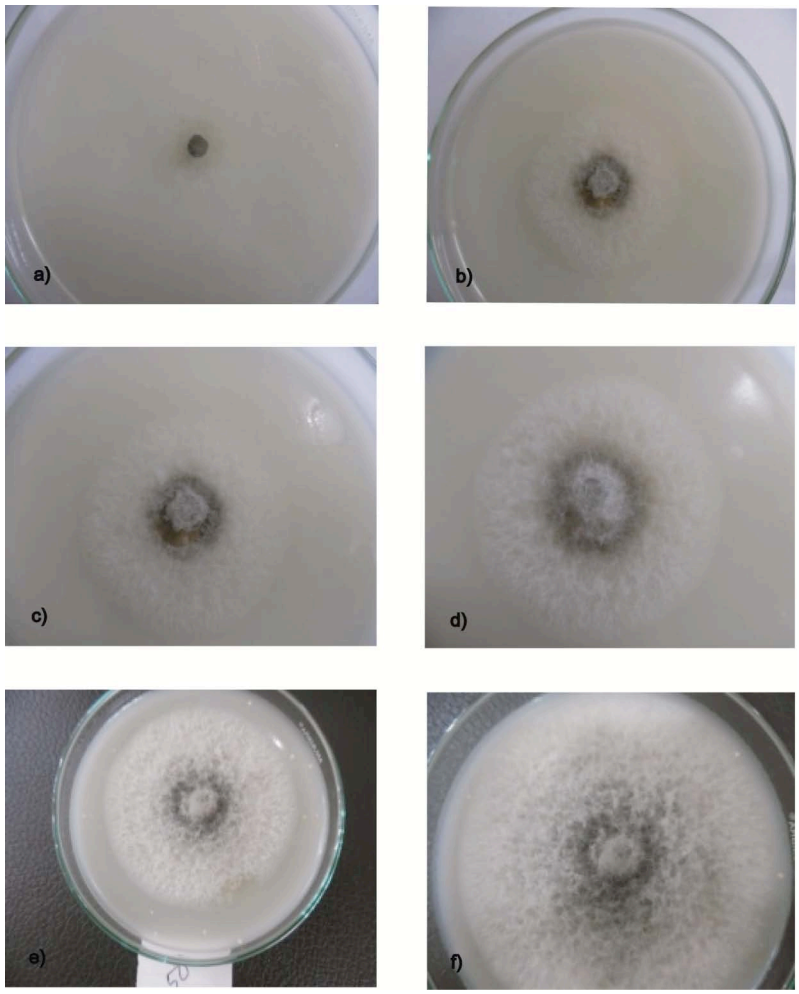


**Figure 6:** Growth of radial mycelia on OMA media for continuous six days: (**a**) day 1, (**b**) day 2, (**c**) day 3, (**d**) day 4, (**e**) day 5, and (**f**) day 6.

**Table 3: Effect of Mycelial Growth on Different Concentrations of Oat Meal Agar (OMA) Media for Eight Continuous Days**

| Media | Mean Mycelial Growth (mm/day) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 | Average |
| 50gm/L | 8.6a | 8.5a | 8.9b | 9.3b | 9.9bc | 9.9b | 10.7b | 10.5b | 9.6b |
| 40gm/L | 8.2a | 7.8b | 8.7b | 9.2b | 9.6c | 9.8b | 10.7b | 10.4b | 9.3b |
| 30gm/L | 7.4a | 7.6b | 8.9b | 9.5b | 10.0bc | 9.9b | 10.7b | 10.8b | 9.3b |
| 20gm/L | 8.4a | 8.6a | 10.2a | 10.4a | 11.1a | 11.1a | 11.2a | 10.6a | 10.2a |
| 10gm/L | 8.4a | 8.7a | 10.1a | 10.1a | 10.6ab | 10.7a | 10.7b | 10.4b | 9.9a |

**Note:** The same letter(s) in column indicate(s) no significant difference at p<0.05.

30g OMA/L (9.3mm) (Table **3**). The results revealed significant (p<0.05) difference of mean mycelial growth (mm/day) among different concentrations of OMA media. Tukey's Post Hoc tests were also revealed the comparison of means of mycelia growth (mm/day) among the concentrations of OMA media (Table **1**). All the five different compositional OMA media were tested for the acervuli production of *C. gloeosporioiedes*. The highest mean acervuli were counted in 30g oat meal/L (3.52 per square cm) and the lowest mean acervuli were in 40 g oat meal/L (2.08 per square cm) followed by 20g (3.48 per square cm), 50g (2.56 per square cm), 10g (2.28 per square cm) (Figure **7**). The results revealed significant difference (p<0.05) of mean acervuli per square cm among the concentration of OMA media (result not shown).

## 4. DISCUSSION

Fungi obtain foods and nutrients from the natural substrates on which they survive [31]. In order to culture the fungi in the laboratory, it is obligatory to provide those fundamental elements and compounds in the medium which are required for their growth and other life process. Neither all media are equally good for all fungi [11]. Identification of the fungus *Colletotrichum* is depending on their unique character setae and conidia [32]. Many scientists studied the growth of *C. acutatum* on CMA, *C. capsici* on PDA, *C. cocodes* on OMA etc. for identification. But there is no report is available on the study of use of different media for the growth and sporulation of *C. gloeosporioiedes* for mango isolates. Hence, different media were tried for the growth and sporulation of *C. gloeosporioiedes*. In the present study, we observed presence of unicellular, hyaline and oval shape conidia as *C. gloeosporioiedes*. Serra *et al*., 2011 [32] observed very similar results from molecular analysis in the identification *C. gloeosporioiedes* isolates from the cashew and mango tree. The present study confirmed the causes of mango diseases under laboratory conditions after inoculation from pathogenecity test. Dinh *et al*. [10] noticed fruits infection by *C. gloeosporioiedes* and anthracnose resistance of some mango cultivar in Thailand.

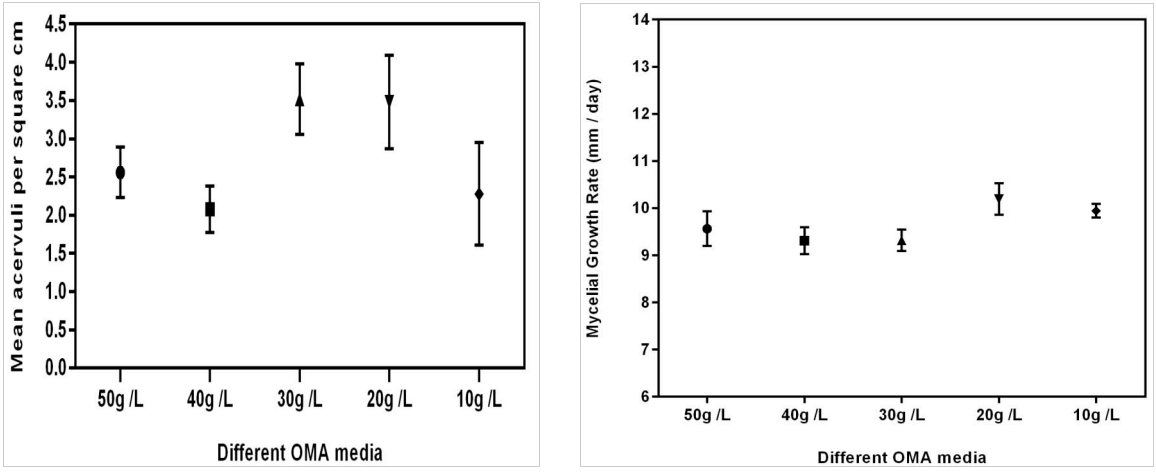


**Figure 7:** Acervulus formation of different concentration on different OMA - Oat Meal Agar media.

The requirement of fungal growth and development during the culture of fungi in pathological laboratory, the essential elements and composition of media should be furnished and adjusted because different media perform differently for different fungi. Therefore, a variety of media were tested for mycelial growth and acervuli development of *C. gloeosporioiedes* [2]. Several authors noticed conflicting results of mean mycelial growth (mm/day) and sporulation of *C. gloeosporioiedes* [2, 34-37]. From the literature, no documents are available for application and identification of suitable media for the mean mycelial growth (mm/day) and sporulation (setae, and conidia) of *C. gloeosporioiedes* in mango leaves and fruits.

In this study mean mycelia growth (mm/day) was maximum in PDA followed MPA and OMA. Naseema *et al*. [33] also noticed maximum growth of *C. gloeosporioiedes* in PDA medium on affected anthurium. Similar results were also found Amarjit *et al*. [34], Jayalakshmi and Seetharaman [35] and Anand and Bhaskaran [36]. They noticed maximum growth in PDA medium. To the contrary, Hubballi *et al*. [2] noticed the highest growth of *C. gloeosporioiedes* in HLEA medium in noni. Nandinidevi [37] noticed maximum growth in anthurium leaf extra medium followed by PDA. This might be due to the variation in nutritional requirement of fungus Hubballi *et al.* [2].

Our results also disclosed mean maximum acervuli per square cm in HLEA followed by GBA but neither PDA nor HLEA is good for culture in the laboratory for the isolation of *C. gloeosporioiedes* from anthracnose affected mango leaves and fruits because they produced no setae. Among the six media only OMA and CMA satisfied for culture in the laboratory for the isolation of *C. gloeosporioiedes* from anthracnose affected mango leaves and fruits but CMA didn't satisfy the mycelial color. It produced white thin mycelial color. Thus, we had only option to select OMA for the isolation of *C. gloeosporioiedes* from anthracnose affected mango leaves and fruits. OMA medium produced 9.5 mean mycelial growth (mm/day) and 1.52 mean acervuli per square cm. The production of mean acervuli per square cm (1.52) was not satisfactory though it satisfied all the sporulation criteria (color, setae, acervuli, and conidia). Thus, we modified the compositional change of OMA to obtain maximum mycelial growth and acervuli production per square cm and the compositional changes satisfied both mean maximum growth (mm/day) and production of mean acervuli production per square cm. Among the five compositions, 20g/L OMA produced 10.2 mean mycelial growth (mm/day) and 3.48 mean acervuli production per square cm with all sporulating characters. At the same time 30g/L OMA produced 9.3 mean mycelial growths (mm/day) and 3.52 mean acervuli production per square cm with all sporulating characters, which were very much satisfactory for isolation of *C. gloeosporioiedes* from anthracnose affected mango leaves and fruits.

## CONCLUSION

The study revealed mean mycelia growth (mm/day) was higher in PDA (Potato dextrose Agar) and mean acervuli per square cm were maximum in HLEA (Host Leaf Extract Agar) but it also produces no setae, which is a very important criterion for effective culture medium. Only OMA (Oat Meal Agar) and CMA (Corn Meal Agar) produced setae, acervuli, and conidia. Therefore, we accepted OMA culture media for future research for the isolation of *C. gloeosporioiedes* from mango anthracnose. Thus, we did research on compositional changes and the study revealed outstanding results. 20g/L OMA produced maximum (10.2mm/day) mean mycelial growth and 3.48 mean acervuli per square cm where as 30g/L OMA produced 9.3 mean mycelial growth (mm/day) and 3.52 mean acervuli per square cm. on the basis of our experiment we prefer 20g/L OMA for future research but anyone can also use 30g/L OMA for future research. These results also conclude that mean mycelial growth (mm/day) and mean acervuli per square cm vary with compositional change of culture media.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests concerning the publication of this paper.

## AUTHORS' CONTRIBUTION

AA and SKA designed the experiment. MA and SKA over all supervised the project. AA collected the samples for the experiments. Manuscript writing and comprehensive manuscript editing and figures developments were done by AA.

## ACKNOWLEDGMENTS

The authors acknowledge the owner of the home garden for providing the access to his garden and to collect the mango leaves and fruits for the study. They also acknowledge the staffs of plant pathology lab of Agrotechnology Discipline, Khulna University, Bangla-desh, for the contribution and support during the

experiments. We also acknowledge HEQEP - Higher Education Quality Enhancement Project (CP: 3447, UGC, World Bank) sub project, Agrotechnology Discipline, Khulna University, Khulna-9208, Bangladesh for financially supports to continue this project.

## REFERENCES

[1] Hayes WB. Fruits Growing in India, 2nd edition, Kitabistan, Allahabad, India, 1953.

[2] Hubballi M, Nakkeeran S, Raguchander T, Anand T and Renukadevi P. Physiological characterisation of Colletotrichum gloeosporioiedes, the incitant of anthracnose disease of noni in India. Archives of Phytopathology and Plant Protection 2011; 44(11); 1105-1114. https://doi.org/10.1080/03235408.2010.482411

[3] Freeman S and Shabi E. Cross-infection of subtropical and temperate fruits by Colletotrichum species from various hosts. Physiol Molecular Plant Pathol 1996; 49; 395-404. https://doi.org/10.1006/pmpp.1996.0062

[4] Reza MMA and Kader KA. Efficacy of pre-harvest field sprays of fungicide on the control of anthracnose and stem end rot of mango. Annual Report, Regional Horticultural Research Institute, Nawabgonj 1995; pp 55-58.

[5] Chowdhury MNA and Rahim MA. Integrated Crop Management to Control Anthracnose (Colletotrichum gloeosporioiedes) of Mango. Journal of Agriculture Rural Development 2009; 7(1 and 2): 115-120.

[6] Fitzell RD and Peak CM. The epidemiology of anthracnose disease of mango: inoculum sources, spore production and dispersal. The Annals of Applied Biology 1984; 104: 53-59. https://doi.org/10.1111/j.1744-7348.1984.tb05586.x

[7] Jeffries P, Dodd JC, Jeger MJ and Plumbley RA. The biology of Colletotrichum species on tropical fruit and vegetables. Plant Pathology 1990; 39: 343-366. https://doi.org/10.1111/j.1365-3059.1990.tb02512.x

[8] Dodd JC, Prusky D and Jeffries P. Fruit diseases. In 'The mango: botany, production and uses'. (Ed.) R. E. Litz, pp. 257–280, (CAB International: Wallingford), 1997.

[9] Arauz LF. Mango anthracnose: economic impact and current options for integrated management. Plant Disease 2000; 84: 600-611. https://doi.org/10.1094/PDIS.2000.84.6.600

[10] Dinh SQ, Chongwungse J, Pongam P and Sangchote S. Fruit infection by Colletotrichum gloeosporioiedes and anthracnose resistance of some mango cultivars in Thailand, Australasian Plant Pathology 2003; 32(4): 533. https://doi.org/10.1071/AP03053

[11] Sanders GM and Korsten L. Comparison of cross inoculation potential of South African avocado and mango isolates of Colletotrichum gloeosporioiedes. Microbiological Research 2003; 158: 143-150. https://doi.org/10.1078/0944-5013-00186

[12] Weir BS, Johnston PR and Damm U. The Colletotrichum gloeosporioiedes species complex. Studies in Mycology 2012; 73: 115-180. https://doi.org/10.3114/sim0011

[13] Abd-AllA MA and Haggag WM. New safe methods for controlling anthracnose disease of Mango (Mangifera indica L.) fruits caused by Colletotrichum gloeosporioiedes (Penz.). Journal of American Science 2010; 8(8): 361-367.

[14] Cai L, Hyde KD, Taylor PWJ, Weir BS, Waller JM, Abang MM, *et al*. A polyphasic approach for studying Colletotrichum. Fungal Diversity 2009; 39: 183-204.

[15] Thinh DC and Kunasakdakul. Inhibition of Colletotrichum gloeosporioiedes and control of postharvest anthracnose disease on mango fruit using propionic acid combined with bee-carnauba wax emulsion. Journal of Agricultural Science 2013; 5(12): 110-116. https://doi.org/10.5539/jas.v5n12p110

[16] Ratanacherdchai K, Wang HK, Lin FC and Soytong K. RAPD analysis of Colletotrichum species causing chilli anthracnose disease in Thailand. Journal of Agricultural Technology 2007; 3(2): 211-219.

[17] Onyeani CA, Osunlaja S, Oworu OO and Sosanya O. First report of fruit anthracnose in Mango caused by Colletotrichum gloeosporioiedes in southwestern Nigeria. International journal of Scientific and Technology Research 2012; 1(4): 30-34.

[18] Jahan N, Sultana S, Adhikary SK, Rahman S and Yasmin S. Evaluation of the growth performance of Trichoderma harzianum (Rifai.) on different culture media. J Agri Vet Sci 2013; 3: 44-50.

[19] Ftayeh RM, Von Tiedemann A and Rudolph KWE. A new selective medium for isolation of Clavibacter michiganensis subsp. michiganensis from Tomato plants and seed. Phytopathology 2011; 101(11): 1355-1364. https://doi.org/10.1094/PHYTO-02-11-0045

[20] Evans AS. Causation and disease: the Henle–Koch postulates revisited. Yale J Biol Med 1976; 49: 175-195.

[21] Giblin FR, Coates LM and Irwin JAG. Pathogenic diversity of avocado and mango isolates of Colletotrichum gloeosporioiedes causing anthracnose and pepper spot in Australia. Australian Plant Pathology 2010; 39(1): 50-62. https://doi.org/10.1071/AP09055

[22] Lakshmi BKM, Reddy P and Prasad RD. Cross-infection potential of Colletotrichum gloeosporioiedes Penz. isolates causing anthracnose in Subtropical fruit crops. Tropical Agricultural Research 2011; 22(2): 183-193. https://doi.org/10.4038/tar.v22i2.2827

[23] Kelemu S, Badel JI, Moreno CX, Miles JW, Chakraborty S, Fernandes CD and Charchar MJDA. Biodiversity, epidemiology and virulence of Colletotrichum gloeosporioiedes. Tropical Grasslands 1997; 31: 387-392.

[24] Admasu W, Sahile S and Kibret M. Assessment of potential antagonists for anthracnose (Colletotrichum gloeosporioiedes) disease of mango (Mangifera indica L.) in North Western Ethiopia (Pawe). Archives of Phytopathology and Plant Protection 2014; 47(18): 2176-2186. https://doi.org/10.1080/03235408.2013.870110

[25] Martínez EP, Hío JC, Osorio LA, Torres MF and Erika P. Identification of Colletotrichum species causing anthracnose on tahiti lime tree, tomato and mango. Agronomia Colombiana 2009; 27(2): 211-218.

[26] Photita W, Taylor PWJ, Ford R, Hyde KD and Lumyong S. Morphological and molecular characterization of Colletotrichum species from herbaceous plants in Thailand. Fungal Diversity 2005; 18: 117-133.

[27] Dhingra OD and Sinclair JB. Basic Plant Pathology Methods. Second Edition. Lewis Publishers, CRC Press inc Florida, USA 1995.

[28] Tasiwal V and Benagi VI. Studies on the cultural and nutritional characteristics of Colletotrichum gloeosporioiedes, the causal organism of papaya anthracnose. Journal of Agricultural Science 2009; 22: 787-789.

[29] Admasu W, Sahile S and Kibret M. Assessment of potential antagonists for anthracnose (Colletotrichum gloeosporioiedes) disease of mango (Mangifera indica L.) in North Western Ethiopia (Pawe). Archives Of Phytopathology And Plant Protection 2014; 47(18): 2176-2186. https://doi.org/10.1080/03235408.2013.870110

[30] Cascino JJ, Harris RF, Smith CS and Andrews JH. Spore yield and microcycle conidiation of Colletotrichum gloeosporioiedes in liquid culture. Applied and Environmental Microbiology 1990; 56(8): 2303-2310.

[31] Phoulivong S, Mckenzie EHC and Hyde KD. Cross infection of Colletotrichum species ; a case study with tropical fruits. Current Research in Environmental and Applied Mycology 2012; 2(2): 99-111.

[32] de SIMR, Serra S, Menezes M, Coelho RSB, Ferraz GMG, Montarroyos AVV, *et al*. Molecular analysis in the differentiation of Colletotrichum gloeosporioiedes isolates from the cashew and mango trees," Brazilian Archives of Biology and Technology 2011; 54(6): 1099-1108.

[33] Naseema A, Nayar K and Gokulapalan C. A new leaf and flower blight of Anthurium andreanum. Journal of Tropical Agriculture 1997; 35: 67.

[34] Amarjit S, Verma KS and Chander M. Effect of different culture media on the growth and sporulation of Colletotrichum gloeosporioiedes causing guava anthracnose. Plant Disease Research 2006; 21: 224.

[35] Jeyalakshmi C and Seetharaman K. Biological control of fruit rot and die-back of chilli with plant products and antagonistic microorganisms," Plant Disease Research 1998; 13: 46-48.

[36] Anand T and Bhaskaran R. Exploitation of plant products and bio-agents for eco-friendly management of Chilli fruit rot disease. Journal of Plant Protection Research 2008; 49(2): 186-197.

[37] Nandinidevi S. Studies on the foliar diseases of anthurium (Anthurium andreanum lind. Ex andre). MSc (Ag) Thesis, Tamil Nadu Agricultural University, Coimbatore, India 2008; 168.

Received on 02-10-2016 Accepted on 07-11-2016 Published on 31-12-2016

DOI: http://dx.doi.org/10.12974/2311-858X.2016.04.02.1