Int.J.Curr.Microbiol.App.Sci (2018) *7*(3): 1578-1584

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 7 Number 03 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.703.189

Efficacy of Indigenous *Trichoderma* Strain Bio-Control against of *Fusarium sp*. Tomato Plant Causal Agent of (*Solanum lycopersicon L.)* *in vitro* Condition

Narayan Prasad Verma*, Yugal Kishor Kuldeep and Bhisham Kumar Sinha

Department of Microbiology & Fermentation Technology, Jacob School of Biotechnology and Bioengineering of Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh-211007, India

**Corresponding author*

Keywords

Trichoderma, Fusarium, Bio-control, Tomato and Antagonistic

Article Info

Accepted:
12 February 2018
Available Online:
10 March 2018

A B S T R A C T

In the present study, *Trichoderma sp*. have been long been used as bio-control agents against tomato fungal disease. In this study, efficacy of the native isolates of *Trichoderma sp*, and another *Trichoderma viride* was procured from Microbial Culture Collection Bank (MCCB-02518), DIM, SHUATS. *Fusarium* wilt is one of the major yield limiting factors in tomato. Results showed *In vitro* efficiency of two species of antagonists (*Trichoderma viride and Trichoderma sp*.) significantly inhibited the mycelia growth of the pathogen. The mycelium inhibition of *Fusarium* was more effective by *Trichoderma viride* (52.31 %) compared to *Trichoderma sp*. (47.09 %).

Introduction

Tomato (*Lycopersicon esculentum* L.) (Miller), new name *Solanum lycopersicom* is one of the most popular and nutritive vegetable crop grown all over the north India. Tomato belongs to the family Solanaceae and is a native crop of Peru and México. This fruit can be eaten raw or cooked. Tomato in large quantities is used to produce soup, juice, ketchup, puree, paste and powder. Tomato is also rich in medicinal values (Chavan *et al.*, 2011). It is reported to have antiseptic properties against intestinal infection. It also contains minerals like iron, phosphorus, It playes an important role in important role in maintaining the human health. Being rich source of lycopene, tomato is used in the treatment of cancer, especially the prostate cancer (Giovannucci, 1999). It is one of the most important nursery-based vegetable crops cultivated for its fleshy fruits. Tomato is a rich source of mineral, vitamins and organic acid, essential amino acids, and dietary fibers. Tomato is known as productive as well as protective food. It is a rich source of vitamin A and C, also contains minerals like iron, phosphorus. Tomato contains lycopene and

Beta carotene pigments (Abdullah *et al.,* 2013). The impotence of tomato and its economic value for farmers, *Fusarium* wilt is one of the common diseases in tomato and the most infections originate from the population associated with infected tomato debris.

The *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the economically most important disease in major tomato growing regions worldwide (Aydi Ben Abdallah *et al.,* 2016). It is a highly destructive pathogen, causing 10 to 50% yield loss in many tomato production areas (Ghazalibiglar *et al.,* 2016). The control of *Fusarium* wilt of tomato is very difficult because pathogen progress within the vascular tissues which limit the effectiveness of fungicides.

Biological control of plant pathogens is considered as a potential control strategy in recent years, because chemical control results in accumulation of harmful chemical residues, which may lead to serious ecological problems. Bio-control mechanisms are likely to be specific for particular antagonists and plant pathogens, and several mechanisms could operate independently or synergistically in any microbial interaction (Joshi *et al.,* 2010).

Probability of biological control of *Fusarium* soil-born root pathogens using the genus *Trichoderma sp.,* The *Trichoderma,* as one of the promising bio-control agent, has been described bio-control agent, has been described (Morsy *et al.,* 2009; Sabalpara *et al.,* 2009). Genus *Trichoderma* has gained immense importance since last few decades due to its biological control ability against several deadly plant pathogens (de Medeiros *et al.,* 2017). Several modes of action have been proposed to explain the bio-control of plant pathogens by *Trichoderma*; these include production of antibiotics and cell wall degrading enzymes, competition for key nutrients, parasitism, and stimulation of plant defence mechanisms and combination of these possibilities (Larkin and Fravel, 1998; Taghdi *et al.,* 2015).

Therefore, the objectives of the present study were to assess the ability of *Trichoderma* sp. in dual culture to inhibit the growth of *Fusarium* sp. *in vitro* condition.

Materials and Methods

Place of work

The experiments were conducted under lab and pots condition in the Department of Microbiology & Fermentation Technology, Jacob School of Biotechnology and Bioengineering of Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh-211007, India during the year 2014-2015.

Collection of pathogenic fungi (*Fusarium sp*) and identification

Fungal pathogen were separated from the infected area (SHUATS) tomato plant sample wilting were collected and brought to the laboratory. Root samples were cut into Approximately 1.5 cm length, surface sterilized for 30-40 seconds. These were placed onto Potato Dextrose Medium (PDA) in Petri-plates and incubated for 7 days at 28+2oC. Fungal isolates appearing on the root pieces were identified and transferred to fresh PDA medium. The pathogens were identified based on the cultural and morphological characters. Morphological characters of the pathogen were studied by slide culture technique. After purification of each of the colonies obtained from single spore or hyphal tip, the detection and identification of *Fusarium* sp. on PDA were performed (Nelson *et al.,* 1983).

Isolation and identification of *Trichoderma*

Species

Trichoderma viride was procured from Microbial Culture Collection Bank (MCCB-02518), DIM, SHUATS. Another species of *Trichoderma* was isolated from the tomato cultivated rhizospheric soils of SHUATS on *Trichoderma* specific medium. Subsequently, all the above isolates were identified on the basis of cultural and morphological characteristics which included colony colour, colony edge, mycelia form, growth pattern, conidia colour, shape and size etc. (Nelson *et al.,* 1983; Saha and Pan, 1997). Collected soil samples were air dried for 4 hour and isolation was done by serial dilution technique. *Trichoderma* Selective Medium (TSH) was used for identification of the isolates of *Trichoderma* (Elad *et al.,* 1983).1 ml of soil suspension was taken with the help of 5ml sterilized pipette and poured on the Petri- plate seeded with TSM. The plates were incubated at 28 + 2^{o}C for 5 days. The purified culture was maintained on PDA slant at 4°C.

In vitro experiment

Antagonistic effect of *Trichoderma* sp. by Dual culture technique

Dual culture method of all the isolates was performed as described by Kamala *et al.,* 2011. It implies evaluation of antagonistic effect of *Trichoderma* sp. against plant pathogens on PDA by measuring their respective radial growth after a certain incubation period. Mycelial plug of 5 mm diameter of *Trichoderma* sp. were placed on PDA plates of about 1 cm each from the corner of the plate. Plant pathogen was placed 4 cm away from the test fungi. Control plates of plant pathogens were also maintained on PDA by placing 5 mm of mycelial plug, without test fungus. The plates were allowed to incubate at 28^{0}C for 5-6 days. Antagonistic effect of the test fungi was estimated by measuring their radial growth in comparison to the control plates by the following formula.

$$I = \frac{(C-T)}{C} \times 100$$

Where,
I: % inhibition in mycelia growth
C: Growth of pathogen in control plates
T: Growth of pathogen in dual culture plates

Statistical analyses

Statistical analysis was performed in order to determine the effect of treatments on observed dual culture. Significance of treatments was tested by One-way Analysis of Variance (ANOVA), and Tukey's HSD test (at $P< 0.05$) was applied for the differences in mean values. All the statistical analyses were completed using SPSS Statistics 20 (IBM, New York, USA).

Results and Discussion

The present study entitled, "Antagonistic effects of *Trichoderma* sp. against *Fusarium* sp. causal agent of tomato wilt" in Allahabad was undertaken at the Biotechnology research Farm, SHUATS, Allahabad. The data so obtained through observation on various aspects were subjected to statistical analysis wherever necessary and the compiled mean data are tabulated in the following pages. Results, thus obtained are presented aspect wise here under:

Morphological identification of *Fusarium* sp.

Primary characteristics On the basis of colony morphology and characteristics of macro and micro conidia, fungal isolates were identified as *Fusarium.*

Fig.1 Effects of *Trichoderma* sp. and *Trichoderma viride* different treatment on Mycelium Inhibition % of *Fusarium* sp. at 120 hours

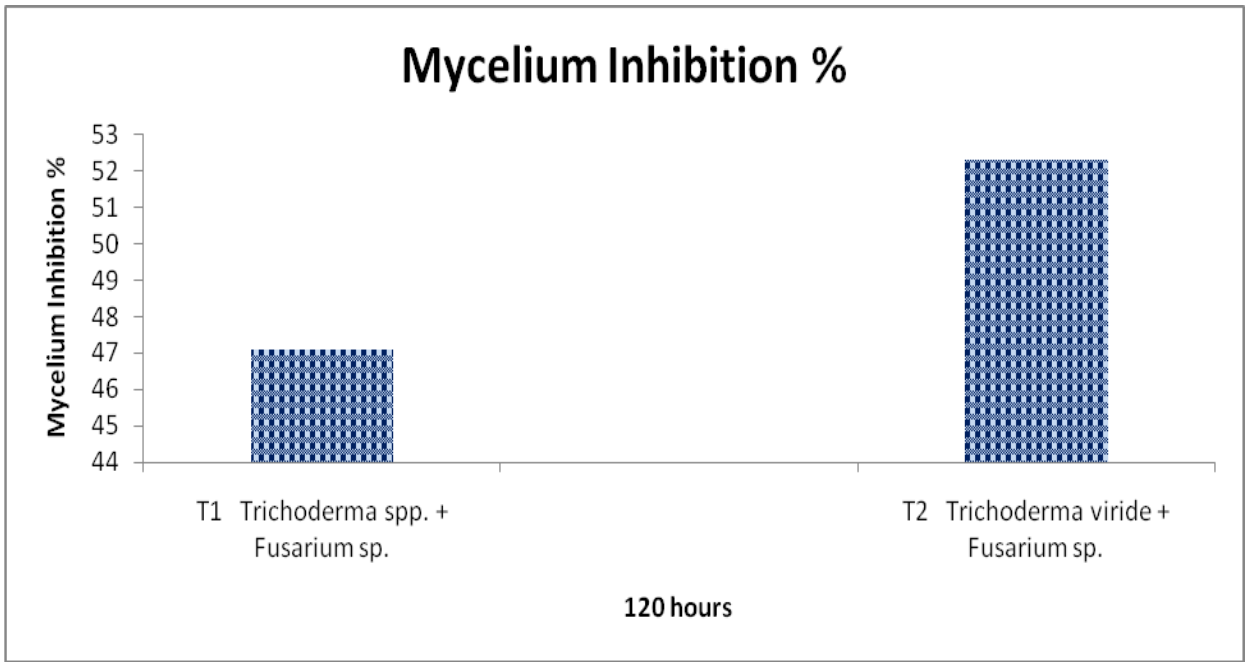
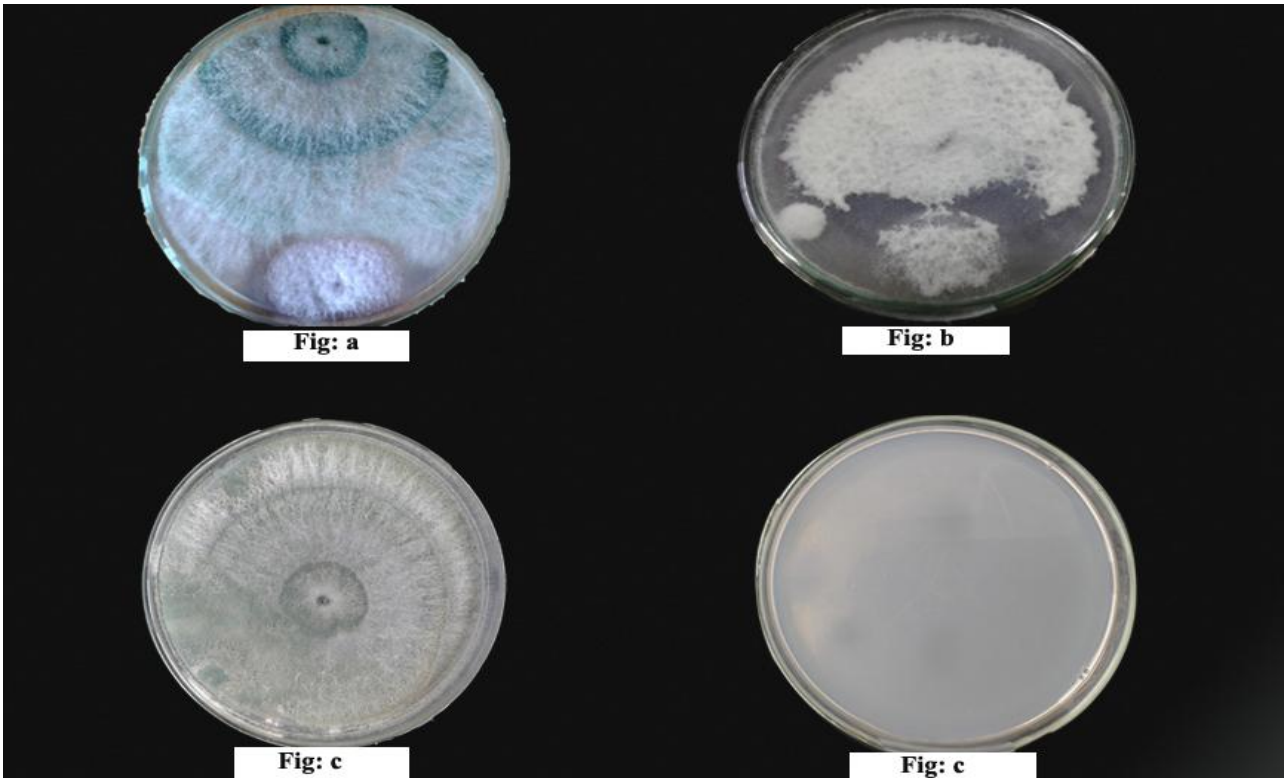
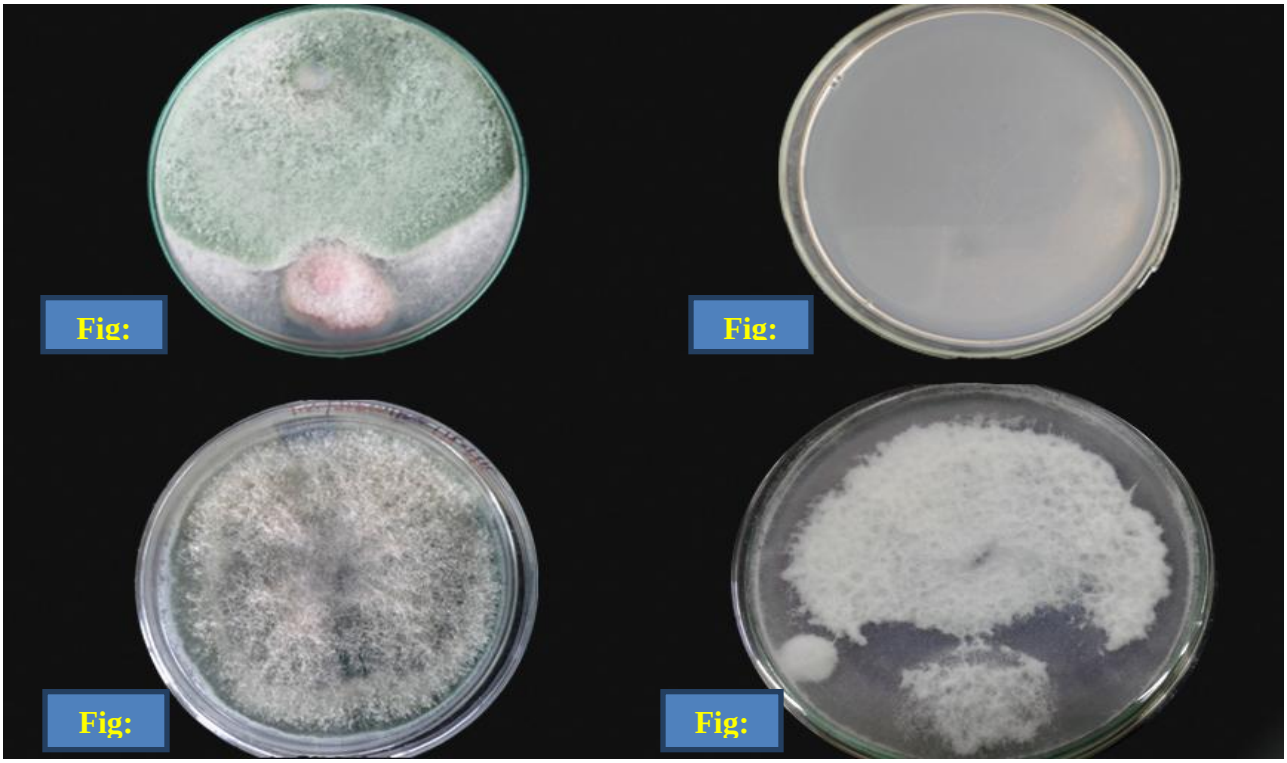


Fig.2 Antagonistic effect of *Trichoderma* spp aginst *Fusarium* spp at 120 houres



A-Trichoderma spp+*Fusarium* spp; B- pure *Fusarium* spp.
C-Pure *Trichoderma* spp. D-Pure media control;

Fig.3 Antagonistic effect of *Trichoderma viride* against *Fusarium* spp at 120 hours



A- *Trichoderma viride+Fusarium* spp at 120 hours; B-Pure media control plate;
C- Colony of *Trichoderma viride;* D- Colony of *fusarium* spp;

On further microscopic study, isolates were identified as *F*. spp. on the basis of macro conidia characteristics which were thin walled generally 3-5 septet and shape of micro conidia, microscopic under identified. Most *Fusarium* specie grow on PDA at 25°C and produce woolly to cottony, flat, spreading colonies. *Fusarium* sp. *is* slow-growing species. The colour and pigmentation of the isolates on PDA medium varied between white, creamish white to creamy, light pink and light purple to violet.

On the basis of the mycelium growth pattern. Some morphological characteristics that were described by Gerlach and Nirenberg (1982) and Nelson *et al.,* (1983). *Trichoderma* colonies grew hastily and readily developed their typical yellow-green color, which aided in their identification from other soil-borne fungi.

Effects of *Trichoderma* sp. and *Trichoderma viride* different treatment on Mycelium Inhibition % of *Fusarium* sp. at 120 hours

The data presented in figure 1 revealed that all the treatment was statistically significant *in vitro* antagonistic activity of undertaken culture of *Trichoderma sp*. significantly and variably reduced the radial colony growth of test pathogen. The reduction of mycelia growth of the pathogen *Fusarium sp* was significantly ($p<0.05$) higher in the dual culture compared with the pathogen control in 120 hours. Our results showed variation in the antagonistic activities of *Trichoderma* sp. isolates against the tested *Fusarium sp*. that inhibition % was seen *Trichoderma viride* showed significantly higher inhibition of the mycelial growth of the pathogen 52.31% than *Trichoderma sp*. 47.09% (Fig. 1). The similar

findings were given by (Patel, 2017). The differences in the mycelial inhibition may be due to the diversity in the *Trichoderma* stains (Sonawane *et al.,* 2015). Reported the dual culture method widely used in antagonistic assay (Arjona-Girona *et al.,* 2014, Sehirli and Saydam 2016). Several experiment s reports indicate that *Trichoderma sp*. can effectively suppress *Fusarium* wilt pathogen in dual culture techniques (Vipul *et al.,* 2016) (Fig. 2 and 3).

Studies showed that the local *Trichoderma* strain can be found the present investigation holds a good promise in tomato wilt management and it showed that *Trichoderma viride* and *Trichoderma* sp. effectively control the *Fusarium* sp. in tomato. However, further studies on effect of these treatments on natural ecosystem need to be undertaken, so that *Trichoderma* can be more effectively utilized in the future and further studies on the effect of these treatments in field conditions need to be undertaken so that *Trichoderma* could be recommended as a biocontrol agent. Since the results of present investigation belong to only one year of experiment, therefore, for reaching to any definite conclusion and recommendation, it needs further conduction of the same trail for at least two successive years in various environment.

References

Abdullah A. 2013. Efficacy of *Trichoderma s*pp. Neem Products and Carbendazim against *Fusarium* Wilt of Tomato in pot condition. Int. J. Agri. Sci. and Res., 3(5): 73-80.

Anitha, A. and Rabeeth, M. 2009. Control of *Fusarium* Wilt of Tomato by Bioformulation of *Streptomyces griseus* in Greenhouse Condition. Afri. J. Basic & Applied Sciences 1(1-2): 9-14.

Arjona-Girona, I., Vinale, F., Ruano-Rosa, D., Lorito, M. and Lopez-Herrera, C.J. 2014. Effect of metabolites from different *Trichoderma* strains on the growth of *Rosellinia necatrix*, the causal agent of avacado white root rot. Euro. J. of Plant Pathology, 140: 385-397.

Aydi Ben Abdallah, R., Jabnoun Khiareddine, H., Nefzi, A., Mokni Tlili, S. and Daami Remadi, M. 2016. Biocontrol of Fusarium wilt and growth promotion of tomato plants using endophytic bacteria isolated from *Solanum elaeagnifolium* stems. J. Phytopath., 164: 811-824.

Chavan, A.A., Deshpande, G.D., Zagade, S.N. 2011. Varietal screening against *Pythium ultimum* Trow by sick soil method in heavy black cotton soil. Asian Journal of Soil Science, 6(1): 74-76.

Elad, Y. and Chet, I. 1981. A Selective medium for improving quantitative isolation of *Trichoderma* spp. From soil. Phytoparasitica. 9(1): 59-67.

Gerlach, W. and Nirenberg. H. 1982. The genus *Fusarium* - A Pictorial Atlas Mitt. Bioi. Bundesanst. Land-Forstwirtsch Berlin-Dahlem. 209: p1-406.

Ghazalibiglar, H., Kandula, D.R.W. and Hampton, J.G. 2016. Biological control of fusarium wilt of tomato by *Trichoderma* isolates. New Zealand PlantProt., 69: 57-63.

Ghazalibiglar, H., Kandula, D.R.W. and Hampton, J.G. 2016. Biological control of fusarium wilt of tomato by *Trichoderma* isolates. New Zealand Plant Prot., 69: 57-63.

Giovannucci, E. 1999. Tomatoes, tomato based products, lycopene and cancer: Review of the epidemiologic literature. Journal of Natural Cancer Institute. 91: 317-331.

Joshi, B.B., Bhatt, R. P. and Bahukhandi, D. 2010. Antagonistic and plant growth

activity of *Trichoderma* isolates of Western Himalayas. Jounral of Enviormental Biology. 31(6): 921-928.

Monte, E. and Liobell, A. 2003. *Trichoderma* in organic agriculture. Proceedings V World Avocado Congress Actas V Congreso Mundial del Aguacate. pp. 725-733

Morsy, M. 2009. Efficiency of *Trichoderma viride* as biocontrol Agent against *Fusarium solani* on Tomato Plants Egypt. Journal of Phytopathology. p 47-57.

Nelson, P.E and Toussoun, T.A. 1983. Marasas WFO. *Fusarium* Species: An Illustrated Manual for Identification. Pennsylvania State University Press; University Park, Pennsylvania. p193.

Patel, 2017. Stuti. Interaction of root colonizing biocontrol agents demonstrates the antagonistic effect against *Fusarium oxysporium* f. sp. *lycopersici* on tomato. European Journal of plant pathology.

Sonawane, A., Mahajan, M. and Renake, S. 2015. Antifungal activity of a fungal isolates against pomegranate wilt pathogen Fusarium. Int. J. Curr. Microbiol. Appl. Sci., 2: 48-57.

Taghdi, Y., Hermosa, R., Domínguez, S., Rubio, M.B., Essalmani, H., Nicolás, C. and Monte, E. 2015. Effectiveness of composts and *Trichoderma* strains for control of *Fusarium* wilt of tomato. Phyto-pathologia Mediterranea, 54: 232.

How to cite this article:

Narayan Prasad Verma, Yugal Kishor Kuldeep and Bhisham Kumar Sinha. 2018. Efficacy of Indigenous *Trichoderma* Strain Bio-Control against of *Fusarium sp*. Tomato Plant Causal Agent of (*Solanum lycopersicon L.) in vitro* Condition. *Int.J.Curr.Microbiol.App.Sci.* 7(03): 1578-1584. doi: https://doi.org/10.20546/ijcmas.2018.703.189