

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.606.286>

## Ability of Antinematic/Antifungal *Bacillus* spp. for the Production of Plant Growth Hormones and Triggering Defense Enzymatic Activities

Tamalika Sarangi<sup>1\*</sup>, S. Ramakrishnan and S. Nakkeeran<sup>2</sup>

<sup>1</sup>Department of Nematology, <sup>2</sup>Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India

\*Corresponding author

### ABSTRACT

The nematicidal/fungicidal indigenous endophytic isolates of *Bacillus weihenstephanensis* (TSB4), *B. cereus* (CLB2), *B. subtilis* (TSB5), *B. cereus* (TSB4D) and *B. licheniformis* (TSB4) were studied for their ability to produce plant growth hormones *in vitro* and to trigger the defence enzymatic activities of tomato under controlled glasshouse/natural sick field conditions. The results of the study distinctly proved that all the isolates of *Bacillus* spp. were capable of producing plant growth hormones *viz.*, indole acetic acid and gibberlic acid and to trigger the defence enzymatic activities of total phenol, peroxidase, polyphenol oxidase and phenyl alanine lyase following *M. incognita* Infestation both under glasshouse and field conditions. Among the five indigenous isolates, the *B. weihenstephanensis* (TSB4) was ranking first in this regard. The biochemical mechanism of the most effective isolate of *B. weihenstephanensis* (TSB4) in triggering the defence enzymatic activities of tomato against *M. incognita* was also confirmed through native-PAGE and SDS-PAGE.

#### Keywords

*Bacillus* spp.,  
*Fusarium oxysporum*  
f.sp. lycopersici,  
Indole acetic acid,  
Peroxidase, Phenol,  
Plant growth  
hormones,  
Polyphenol oxidase.

#### Article Info

Accepted:  
29 May 2017  
Available Online:  
10 May 2017

### Introduction

Biochemical changes due to nematode infestation and their antagonists in plant system are well documented by several authors (Ganguly and Dasgupta, 1979). Information on this line is much useful to know the biochemical mechanism of bioagents used for the management of phytonematodes.

The biochemical changes induced by *B. subtilis*, *B. firmus* and *B. coagulans* reported to be effective against *M. javanica* in

eggplants indicated that all three species of *Bacillus* were capable of accumulating phenolic compounds and enhancing defence enzymatic activities of peroxidase, phenyl alanine lyase, catalase, ascorbate peroxidase and declining super oxide dismutase (Abbasi *et al.*, 2014).

In addition the plant health could be improved by controlling a range of plant pathogens including bacteria, fungi and nematodes. *Bacillus* spp. are known to enhance plant

growth and health through their direct or indirect mechanisms. The use of PGPR like *Bacillus* spp. recently named as plant probiotics to control plant pathogens is receiving increasing attention as they may represent an alternative approach to chemical pesticides (Shadia, 2013). In this regard Tamalika Sarangi (2014) identified native isolates of *Bacillus* spp. from Tamil Nadu, India effective against *M.incognita* and *Fusarium oxysporum f.sp.lycopersici* in continuation the present study was initiated to understand the ability of identified *Bacillus* spp. for the production of plant growth hormones and to trigger the plants defence enzymatic activities against root knot nematode under controlled glasshouse and natural sick field conditions in tomato using biochemical analysis and molecular techniques.

## Materials and Methods

### Indole Acetic Acid (IAA)

Five isolates of *Bacillus* spp. viz., *B. weihenstephanensis* (TSB4), *B. cereus* (CLB2D), *B. subtilis* (TSB5), *B. cereus* (TSB4D), *B. licheniformis* (TSB3) showing promising inhibitory effect on *M.incognita* and *Fusarium oxysporum f.sp.lycopersici* were grown in nutrient agar (NA) medium for 48 h. Then the cultures were incubated at 28±2°C for 7 days. After the incubation period, the culture was centrifuged at 10000 rpm for 10 min to remove the bacterial cells and the supernatant was brought to pH 2.8 using 1N HCl and 50 ml of acidified supernatant was taken in 100 ml conical flask and equal volume of diethyl ether was added and incubated in dark for four hour.

The extraction of IAA was performed at 4<sup>0</sup> C through separating funnel using diethyl ether. The aqueous phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, 7 ml methanol

was added and the IAA present in the methanol extract was determined using the method of Gorden and Paley (1957). To 5 ml of methanol extract, 1.0 ml of distilled water and 4 ml of salper's reagent (1.0 ml of FeCl<sub>3</sub> in 50 ml of 35% perchloric acid) were added and incubated in dark for an hour. The intensity of pink colour developed was read at 545 nm in spectrophotometer. From the standard curve prepared with known concentration of IAA, the quantity of IAA in the culture filtrate was calculated (Lwin *et al.*, 2012).

### Gibberlic acid (GA)

The above *Bacillus* isolates (Table 1) cultures were grown in NA medium for 48 h. The culture was incubated at 28±2°C for seven days. After incubation period, the culture was centrifuged at 10000 rpm for 10 min and the supernatant collected. Then pellet was re-extracted with phosphate buffer (pH 8.0) and again centrifuged as above. Both the supernatant were pooled and acidified to pH 2.5 using 5N HCl and partitioned with equal volume of ethyl acetate for five times. The ethyl acetate extract dried at 32°C and the residue was dissolved in 2 ml distilled water containing 0.05% of Tween 80. The blank sample was treated with 5% HCl and the absorbance of the sample as well as blank was measured at 254 nm in a spectrophotometer to quantify the content of GA (Richardson *et al.*, 2009).

### Biochemical changes induced by isolates of *Bacillus* spp. in tomato challenged with *M. incognita*

Biochemical analysis was made with tomato root samples collected from the above experiments meant for the management of *M. incognita* under controlled glasshouse conditions and field conditions. Tomato plants from glasshouse and field conditions were carefully uprooted without causing

damage to root tissues at different intervals (Tamalika Sarangi, 2014).

### **Estimation of total phenol**

The procedure developed by Malik and Singh (1980) was followed for the estimation of total phenol from the plant samples collected in the present study. One g root sample was ground in 10 ml of 80 per cent ethanol using pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 20 min. The supernatant dried and dissolved in 5 ml distilled water. The aliquots (2 ml) taken in test tubes were made to the volume of 3 ml with water and 0.5 ml of Folin-Ciocalteu reagent. After three min two ml of 20 per cent  $\text{Na}_2\text{CO}_3$  was added to each tube and placed in boiling water for a min and cooled. The absorbance was measured at 650 nm.

### **Estimation of peroxidase ( $\text{P}_2\text{O}_2$ )**

The  $\text{P}_2\text{O}_2$  activity was assayed spectrophotometrically (Hartee, 1955). Reaction mixture consists of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% hydrogen peroxide. The reaction mixture was incubated at room temperature ( $28 \pm 2^\circ\text{C}$ ). The change in absorbance at 420 nm was recorded at 30 sec intervals for three min. Boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance of the reaction mixture per min on fresh weight basis (Hammerschmidt and Kue, 1982).

### **Estimation of polyphenoloxidase (PPO)**

The PPO activity was determined following the procedure as described by Byrant and Forrest (1979). The enzyme extract was prepared by homogenising one g root tissue in 100 ml aliquots of cold acetone. The homogenate was filtered through Whatman No.1 filter paper and air dried. The resulting dry powder was used for the estimation of

activity of PPO. One g dry powder prepared was ground with two successive 20 ml aliquots of 25 mM phosphate buffer (pH 6.2) in a mortar chilled in an ice bath. Then filtered through Whatman No.1 filter paper and diluted to 50 ml with phosphate buffer. Each two ml of phosphate buffer and enzyme extract was taken in a test tube and to this 1 ml of para coumaric acid and 1 ml of manganese chloride were added and incubated in dark at  $30^\circ\text{C}$ . Before and after 50 min of incubation two ml of the mixture, were taken and 5.2 ml of perchloric acid and 0.5 ml of ferric nitrate solution were added and diluted to 10 ml with water. After incubation period of 60 min in dark the absorbance was measured at 535 nm.

### **Estimation of phenylalaninylase (PAL)**

One gram of plant sample was homogenized in 3 ml. of ice cold 0.1M sodium borate buffer, pH 7.0, containing 1.4 mM of 2-mercaptoethanol and 50 mg of insoluble polyvinylpyrrolidone. The resultant extract filtered through cheese cloth and the filtrate was centrifuged at 15,000 rpm for 15 min at  $4^\circ\text{C}$  and the supernatant was used as the enzyme source. The PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.4ml of enzyme extract was incubated with 0.5 ml of 0.1M borate buffer (pH 8.8) and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at  $30^\circ\text{C}$ . The amount of trans cinnamic acid synthesized was calculated using its extinction coefficient of  $9630 \text{ M}^{-1}\text{cm}^{-1}$  (Dickerson *et al.*, 1984). The enzyme activity was expressed on fresh weight basis as  $\text{nmol trans-cinnamic acid min}^{-1} \text{ mg}^{-1}$  of sample.

### **Native – PAGE analysis**

The isoform profiles of PO and PPO were viewed through native polyacrylamide gel electrophoresis (Native – PAGE) as described

by Laemmli and Favre. (1970). The proteins extract was prepared by homogenizing one g root samples in 2 ml of 0.1 M sodium phosphate buffer (pH 7.0) and centrifuged at 16,000 rpm for 20 min at 4°C and the content of the protein was determined using standard method (Bradford,1976).

The samples of 50 µg protein were loaded on eight per cent polyacrylamide gel. After electrophoresis the PO isoforms were visualized by soaking the gels in staining solution containing 0.05 per cent benzidine and 0.03 per cent H<sub>2</sub>O<sub>2</sub> in acetate buffer (20 mM; pH 4.2) as per the method of Naddlon and Sequira, (1980). For assessing PPO isoform profiles, the gels were equilibrated for 30 min in 0.1 per cent p - phenylene diamine followed by addition of 10 mM catechol in the same buffer (Jayaraman *et al.*, 1987).

### **Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE)**

One g powdered root sample was extracted with one ml of sodium phosphate buffer (pH 7.0) under 4°C. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was used for the SDS – PAGE (Laemmli and Favre. 1973). Forty µg of proteins from different treatments was taken and mixed with 10 µl of the sample buffer in a microfuge tube, boiled for 4 min and incubated at 4°C for 30 min. Then the samples containing equal amounts of protein were loaded into the wells of polyacrylamide gels (Sigma – Aldrich Techware system, Sigma, USA). The medium range molecular weight markers (Bangalore Gennei, India) were used throughout the experimentation and the electrophoresis was carried out at constant voltage of 75 volts for 2 h. The gels were stained with 0.2 per cent coomassie brilliant blue (R 250) solution.

## **Results and Discussion**

### **Ability of isolates of *Bacillus* spp. for the production of plant growth hormones**

The most effective first five ranking different isolates of endophytic *Bacillus* spp. were tested for the production of most important plant growth hormones of IAA and GA.

#### **IAA**

All the above five endophytes were capable of producing the IAA and it was in higher side with *B. weihenstephanensis* (TSB4) (1400 ng/ml) compared to other species / isolates of CLB2 of *B. cereus* (1325 ng/ml), TSB5 of *B. subtilis* (750 ng/ml), TSB4D of *B. cereus* (460 ng/ml) and TSB3 of *B. licheniformis* (600 ng/ml). The production rate of IAA was differing significantly among the species/isolates tested (Table 1).

#### **GA**

As above the production rate of GA in different species/isolates was differing significantly with each other and the above trend of highest production of 454 ng/ml GA was noticed with *B. weihenstephanensis* (TSB4). It was lower and ranging from 198 to 273 ng/ml in the remaining other endophytes of *B. cereus* (CLB2), *B. subtilis* (TSB5), *B. cereus* (TSB4D) and *B. licheniformis* (TSB3) (Table 1).

Kumar *et al.*, (2011) stated that the *Bacillus* spp. could act as biofertilizer and / or antagonists (biopesticides) or simultaneously both in general. In particular the mechanisms of growth promotion due to multiple species of *Bacillus* were attributed to production of growth stimulating phytohormones, solubilisation and mobilisation of phosphate, siderophore production and antibiosis, i.e., production of antibiotics, inhibition of plant

ethylene synthesis and induction of plant systemic resistance to pathogens. The similar view point put forth by Richardson *et al.*, (2009), Idris *et al.*, (2007), Gutierrez- Manero *et al.*, (2001) and Whipps *et al.*, (2001).

It is also very likely the growth promoting effects of plant growth promoting rhizobacteria (PGPR) are due to their production of plant growth regulators such as IAA, gibberellin, cytokinin (Bottini *et al.*, 2004; Bloemberg *et al.*, 2001). In continuation it is stated that there was production of gibberellin by *B. pumilus* and *B. licheniformis* (Gutierrez – Manero *et al.*, 2001) and IAA by *B. subtilis* and *B. amyloliquefaciens* (Idris *et al.*, 2004).

### **Biochemical mechanisms of different isolates of *Bacillus* spp. in the management of *M. incognita* in tomato under different conditions**

#### **Under glasshouse conditions**

Tomato plants inoculated with nematodes and treated with different isolates of *Bacillus* spp. were subjected to the following biochemical mechanisms or changes in comparison of uninoculated and untreated plants. The results of the study furnished below.

#### **Total phenol content**

The total phenol content in plants treated with all the isolates of *B. weihenstephanensis* (TSB4), *B. cereus* (CLB2D), *B. subtilis* (TSB5), *B. cereus* (TSB4D) and *B. licheniformis* (TSB3) increased compared to uninoculated and untreated control in the experiments conducted for the management of *M. incognita* in tomato under controlled conditions.

The phenol content observed was highest with *B. weihenstephanensis* (TSB4) @ 5 g/plant

which is found to be significantly effective for the management of *M. incognita* in tomato (Tamalika Sarangi, 2014) and it was 260.20  $\mu\text{g}^{-1} \text{mg}^{-1}$  over uninoculated (96.50  $\mu\text{g}^{-1} \text{mg}^{-1}$ ) and healthy control plants (102.29  $\mu\text{g}^{-1} \text{mg}^{-1}$ ). The effect of increase in phenol content as defence mechanism caused by *B. weihenstephanensis* (TSB4) was followed by the other isolates of CLB2D of *B. cereus*, TSB5 of *B. subtilis*, TSB4D of *B. cereus* and *B. licheniformis*. The phenol content increased by the above endophytes is differing significantly among each other as well as from uninoculated and healthy plants (Table 2).

#### **PO**

The defence enzymatic activity of PO in tomato inoculated with *M. incognita* and treated with different isolates of *Bacillus* spp. was found to be highest with the *B. weihenstephanensis* (TSB4) (1.957  $\mu\text{g}$  of catechol /g of root tissues). The trend was similar with other isolates of *Bacillus* spp. showing the enhanced activity of peroxidase ranging from 0.853 to 1.192 compared to uninoculated (0.108) and healthy plants (0.154). With regard to increase in peroxidase activity in relation to days after inoculation it is observed that the defence enzymatic activity is increasing continuously up to 3 days after inoculation (DAI) and declining thereafter in all the five endophytes (Table 3).

#### **PPO**

The defence enzymatic activity of PPO in tomato inoculated with *M. incognita* and treated with different isolates of *Bacillus* spp. was found to be highest with the *B. weihenstephanensis* (TSB4) (1.610). The trend was similar with other isolates of *Bacillus* spp. showing the enhanced activity of PPO ranging from 0.230 to 0.980 compared to uninoculated (0.270) and healthy plants (0.225) (Table 4).



## **PAL**

The defence enzymatic activity of PAL in tomato inoculated with *M. incognita* and treated with different isolates of *Bacillus* spp. was found to be highest with the *B. weihenstephanensis* (TSB4) (0.758). The trend was similar with other isolates of *Bacillus* spp. showing the enhanced activity of PAL ranging from 0.175 to 0.310 compared to uninoculated (0.337) and healthy plants (0.306).

With regard to increase in PAL activity in relation to days after inoculation it is observed that the defence enzymatic activity is increasing continuously upto 3 DAI and declining thereafter in all the five endophytes found to be effective against *M. incognita* (Table 5).

## **Under field conditions**

The most effective isolate of *B. weihenstephanensis* (TSB4) among the five indigenous isolates of *Bacillus* spp. was experimented with different dosage of 1 to 5 kg /ha for the management of *M. incognita* in tomato under field conditions (Tamalika Sarangi, 2014). The root samples were assayed for the biochemical changes and results furnished below.

## **Total phenol content**

There was significant difference in total phenol content in roots of tomato followed by the application of *B. weihenstephanensis* (TSB4). The highest increase in total phenol content (287.99 mg / g root) was registered by the highest dosage of *B. weihenstephanensis* (TSB4) @ 5 kg/ha and it was on par with the lesser dosage of 4 kg / ha (255.80 mg/g root). The trend of increase in total phenol content with increase in the dosage of *B. weihenstephanensis* (TSB4) was noticed (Table 6).

It is a well-known fact that phenolic compounds play a major role in the defence mechanism of plants against various external and infectious agents. A distinct correlation between the degree of plant resistance and phenolics present in plant tissue has been observed by several authors (Patidar *et al.*, 2013; Ramamorthy *et al.*, 2001; Doley and Paramijit, 2013; Troll and Rhode, 1966 and Brueske and Dropkin, 1972).

The results of the present investigations revealed that there was significant increase in the total phenol content of the tomato plants infected with *M. incognita* and treated with isolates of *Bacillus* spp. both under controlled glasshouse and field conditions. There was positive correlation between increase in phenol content and the dosage of *Bacillus* spp. used in the present study.

The phenol is providing resistance to nematodes in plants either by repelling the juveniles or by adversely affecting the development of nematode juveniles according to Singh *et al.* (1983). Accumulation of phenols in plant system is imparting resistance to invading plant pathogens including nematodes. The use of many biocontrol agents including plant growth promoting rhizobacteria like *Bacillus* spp. resulted in accumulation of phenol as biochemical changes in favour of plants and against invading pathogens like nematodes (Pitcher *et al.*, 1989).

## **PO**

There was significant increase in peroxidase activity with increase in the dosage of *B. weihenstephanensis* (TSB4). The highest peroxidase activity with 1.349 min /g root was registered by the highest dosage of *B. weihenstephanensis* (TSB4) @ 5 kg /ha with 63.63 per cent increase over untreated control.

The other dosage of *B. weihenstephanensis* (TSB4) from 1 to 4 kg / ha also had effect to increase the peroxidase activity and it was ranging from 13.63 to 50 per cent over untreated control and differed significantly with each other (Table 6).

Increase in defence enzymatic activity of peroxidase was observed in *M. incognita* infected plants of tomato treated with *Bacillus* spp. both under controlled and field conditions. The invasion of nematodes into plant roots appears to increase the activity of peroxidase in infected roots of host (Ibrahim, 1991). Mohanty *et al.*, (1986) also reported similar trend in peroxidase activity in resistant cowpea plants inoculated with *M. incognita*. The higher activity of peroxidase enzyme was detected in *M. incognita* resistant tobacco and tomato tissues by Shukla and Charaborty (1988). It was speculated that reduced levels of peroxidase activity could be one of the reasons for susceptibility to nematodes (Ganguly and Dasgupta, 1979). In the present study also low level of peroxidase was observed in the untreated plant whereas it was higher in plants treated with *Bacillus* spp. Voluminous literature on this line is already documented by earlier workers (Akram *et al.*, 2011 and Ramanujam *et al.*, 2012).

### **PPO**

There was significant enhancement in PPO activity of tomato following the soil application of *B. weihenstephanensis* (TSB4) for the management of *M. incognita*. The increase in PPO activity in different dosage of *B. weihenstephanensis* (TSB4) showed direct relationship between increase in PPO activity and the dosage of *B. weihenstephanensis* (TSB4). The highest PPO activity of 1.520 µg/g was recorded in the treatment of *B. weihenstephanensis* (TSB4) @ 5 kg/ha as against 0.138 µg/g in untreated control (Table 6).

There was triggering in the defence enzymatic activity of PPO in *M. incognita* infected plants of tomato treated with *Bacillus* spp. both under controlled and field conditions. Ramanujam *et al.*, (2012) reported that three isolates of *B. subtilis* viz. EXB-123, ENB- 24 and S-9 increasing the activities of polyphenoloxidase proved to be promising bacterial antagonists against chilli anthracnose pathogen, *Colletotrichum capsici*.

### **PAL**

There was significant enhancement in PAL activity of tomato following the soil application of *B. weihenstephanensis* (TSB4) evaluated for the management of *M. incognita*. The increase in PAL activity at different dosage of *B. weihenstephanensis* (TSB4) showed direct relationship between increase in PPO activity and the dosage of *B. weihenstephanensis* (TSB4). The highest PPO activity of 0.632 µg/g was recorded in the treatment of *B. weihenstephanensis* (TSB4) @ 5 kg/ha as against 0.154 µg/g in untreated control (Table 6). As in the case of other defence enzymatic activity there was increase in *M. incognita* infected plants of tomato treated with *Bacillus* spp. both under controlled and field conditions. The present experimental results was confirmed by Abbasi *et al.*, (2014) who mentioned that the biochemical changes viz. accumulation of phenols and enhancement of defence enzymatic activities of PO and PAL induced by *B. subtilis*, *B. firmus* and *B. coagulans* were responsible for their effectiveness against *M. javanica* in eggplants.

### **Confirmation of defense enzymatic activities of PO and PPO in tomato through Native – PAGE**

#### **PO**

The results of Native–PAGE analysis displayed different banding pattern with the

protein sample of tomato plants challenged with *M. incognita* and treated with talc formulated most effective five isolates of *Bacillus* spp. (Tamalika Sarangi, 2014) compared to healthy and inoculated control. The study indicated that there was definite induction of defence enzymatic activity of PO through displaying five different banding patterns denoted as PO1, PO2, PO3 and PO4.

The *B. weihenstephanensis* (TSB4) identified as the most effective endophyte with antinemic and antifungal property coupled with plant growth promoting ability among the five isolates of *Bacillus* spp. showed higher number of bands (4 nos) compared to other species / healthy and inoculated control plants. Therefore the study inferred that the *B. weihenstephanensis* (TSB4) enhance the defence enzymatic activity of PO as biochemical mechanism in the management of *M. incognita* in tomato (Plate 1).

Although the tomato plants challenged with *M. incognita* and bacterized with *B. weihenstephanensis* (TSB4) displayed the presence of four bands of peroxidase as PO1 to PO 4, the isoform was observed only in respect of PO4 in the present study. No

information is available to compare the enhancement of defence enzymatic activity of PO following the application of *B. weihenstephanensis* (TSB4) in tomato challenged with *M. incognita*. However the induction of PO activity observed by Bradly *et al.* (1992) in cucurbit, tobacco, wheat and rice crops due to *B. subtilis* strains BsW2 and BsM3 supported the present findings.

**PPO**

As above the tomato plants challenged with *M. incognita* and treated with talc *B. weihenstephanensis* (TSB4) displayed different bands for indicating the prominent activities of PPO in Native PAGE. The bands were designated as PPO1, PPO2, PPO3, PPO4 and PPO5 (Plate 1). But the induction of enzymatic activity of PPO was observed to be least in untreated control plants inoculated with *M. incognita*. Hence the triggering of defence enzymatic activity of PPO is confirmed in tomato challenged with *M. incognita* and coinoculated with *B. weihenstephanensis* (TSB4) as a measure to contain root knot nematode disease.

**Table.1** Production of Indole 3-acetic acid and Gibberellic acid by different isolates of *Bacillus* spp

S. No	<i>Bacillus</i> spp.	Isolates	IAA (ng/ml)	GA (ng/ml)
1	<i>B. weihenstephanensis</i>	TSB4	1400 (37.41)	454 (21.30)
2	<i>B. cereus</i>	CLB2D	1325 (36.40)	273 (16.52)
3	<i>B. subtilis</i>	TSB5	750 (27.38)	237 (15.39)
4	<i>B. cereus</i>	TSB4D	600 (24.49)	250 (15.81)
5	<i>B. licheniformis</i>	TSB3	460 (21.44)	198 (14.07)
6.	Untreated control		36 (6.03)	49 (7.09)
	SEd		9.63	5.75
	CD (P=0.05)		24.76	22.54

Note: The figures in parentheses are square root transformed values.



**Table.2** Content of total phenols in plants treated with different isolates of *Bacillus* spp. and challenged with *M. incognita* under glasshouse conditions\*

S.No.	Species/ isolates	Phenol content (changes in µg of catechol / g of root tissues) after different days of inoculation (DAI)					
		0 DAI	1 DAI	2 DAI	3 DAI	4 DAI	5 DAI
1	<i>B. weihenstephanensis</i> (TSB4)	123.11 (11.09)	169.69 (13.02)	224.39 (14.97)	291.90 (17.08)	237.44 (15.40)	206.93 (14.38)
2	<i>B. cereus</i> (CLB2D)	118.15 (10.98)	142.93 (11.95)	203.55 (14.26)	262.20 (16.19)	211.68 (14.54)	202.96 (14.24)
3	<i>B. subtilis</i> (TSB5)	110.02 (10.48)	132.67 (11.51)	183.39 (13.54)	220.60 (14.85)	194.07 (13.93)	176.36 (13.27)
4	<i>B. cereus</i> (TSB4D)	101.56 (10.07)	113.36 (10.64)	163.04 (12.70)	191.45 (13.83)	173.42 (13.16)	161.44 (12.70)
5	<i>B. licheniformis</i> (TSB3)	96.60 (9.82)	103.15 (10.15)	123.26 (11.10)	155.50 (12.46)	177.66 (13.32)	121.50 (11.02)
6	Healthy control (inoculated)	64.00 (8.00)	75.64 (8.69)	87.22 (9.33)	96.50 (9.82)	92.30 (9.60)	81.39 (9.02)
7	Control	66.50 (8.15)	87.58 (9.35)	95.33 (9.76)	102.29 (10.11)	97.32 (9.86)	91.62 (9.57)
	SEd	0.86	1.23	1.53	2.77	3.05	3.57
	CD	1.74	2.54	3.32	4.33	4.79	5.00

Note: Figures in parentheses are square root transformed values.

\* All the values are pooled data of two identical experiments.

**Table.3** Enzymatic activity of peroxidase in tomato treated with different isolates of *Bacillus* spp. and challenged with *M. incognita* under glasshouse conditions\*

S.No.	<i>Bacillus</i> spp.	Peroxidase activity (changes in absorbance min <sup>-1</sup> g <sup>-1</sup> of root tissues) days after inoculation (DAI)					
		0	1	2	3	4	5
1	<i>B. weihenstephanensis</i> (TSB4)	0.888 (0.94)	1.076 (1.03)	1.428 (1.19)	1.957 (0.82)	1.839 (1.35)	1.742 (1.31)
2	<i>B. cereus</i> (CLB2D)	0.853 (0.92)	1.032 (1.01)	1.366 (1.16)	1.943 (1.39)	1.821 (1.34)	1.702 (1.30)
3	<i>B. subtilis</i> (TSB5)	0.742 (0.80)	0.949 (0.97)	1.408 (1.18)	1.685 (1.29)	1.511 (1.22)	1.506 (1.22)
4	<i>B. cereus</i> (TSB4D)	0.835 (0.91)	0.933 (0.96)	1.335 (0.57)	1.618 (1.27)	1.480 (1.21)	1.441 (1.20)
5	<i>B. licheniformis</i> (TSB3)	0.687 (0.82)	0.820 (0.90)	1.200 (1.09)	1.334 (1.15)	1.275 (1.12)	1.192 (1.09)
6	Healthy control	0.089 (0.29)	0.092 (0.30)	0.098 (0.31)	0.108 (0.32)	0.101 (0.39)	0.098 (0.31)
7	Control	0.098 (0.29)	0.102 (0.31)	0.146 (0.37)	0.154 (0.39)	0.148 (0.38)	0.156 (0.39)
	SEd	0.07	0.09	0.04	0.05	0.03	0.04
	CD (P=0.05)	0.16	0.18	1.69	1.30	0.54	0.07

Note: Figures in parentheses are square root transformed values.

\* All the values are pooled data of two identical experiments.

**Table.4** Enzymatic activity of polyphenoloxidase (PPO) in tomato treated with different isolates of *Bacillus* spp. and challenged with *M. incognita* under glasshouse conditions \*

S.No.	<i>Bacillus</i> spp.	Polyphenoloxidase activity (changes in absorbance min <sup>-1</sup> g <sup>-1</sup> of root tissues) days after inoculation (DAI)					
		0	1	2	3	4	5
1	<i>B. weihenstephanensis</i> (TSB4)	0.240 (0.48)	0.940 (0.96)	1.205 (1.09)	1.610 (1.26)	1.520 (1.23)	1.330 (1.15)
2	<i>B. cereus</i> (CLB2D)	0.230 (0.47)	0.930 (0.96)	1.169 (1.08)	1.530 (1.23)	1.490 (1.22)	1.320 (1.14)
3	<i>B. subtilis</i> (TSB5)	0.223 (1.10)	0.825 (0.90)	1.089 (1.04)	1.415 (1.18)	1.305 (1.14)	1.200 (1.09)
4	<i>B. cereus</i> (TSB4D)	0.228 (0.47)	0.810 (0.90)	1.048 (1.02)	1.395 (1.18)	1.270 (1.12)	1.168 (1.00)
5	<i>B. licheniformis</i> (TSB3)	0.209 (0.45)	0.645 (0.80)	0.989 (0.99)	1.289 (1.13)	1.100 (1.04)	0.980 (0.98)
6	Healthy control	0.125 (0.35)	0.139 (0.37)	0.205 (0.45)	0.225 (0.47)	0.215 (0.46)	0.238 (0.50)
7	Control	0.140 (0.37)	0.155 (0.39)	0.245 (0.49)	0.270 (0.51)	0.260 (0.50)	0.255 (0.50)
	SEd	0.03	0.04	0.05	0.05	0.04	0.04
	CD (P=0.05)	0.05	0.25	1.69	1.30	0.54	0.07

Note: Figures in parentheses are square root transformed values.

\* All the values are pooled data of two identical experiments.

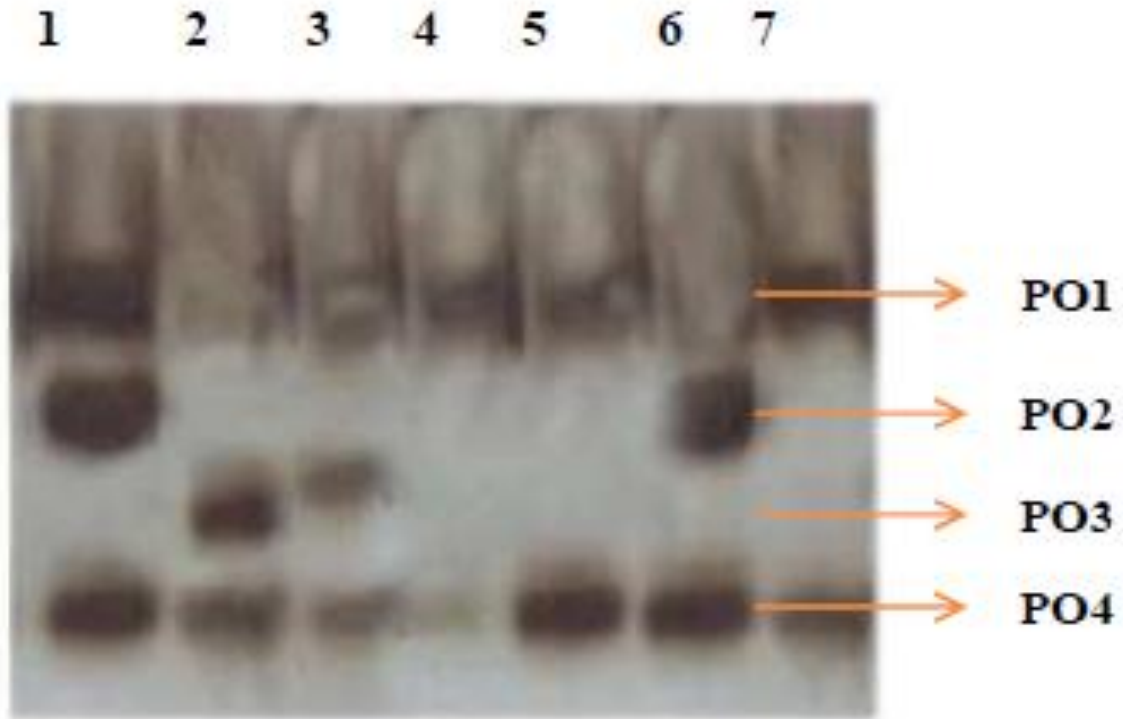
**Table.5** Enzymatic activity of phenylalaninelyase (PAL) in tomato treated with different isolates of *Bacillus* spp. and challenged with *M. incognita* under glasshouse conditions \*

S.No.	Isolates	PAL activity (changes in $\mu$ mol of cinnamic acid/ min/g of root tissues) days after inoculation (DAI)					
		0	1	2	3	4	5
1	<i>B. weihenstephanensis</i> (TSB4)	0.183 (0.42)	0.292 (0.54)	0.497 (0.70)	0.758 (0.87)	0.502 (0.70)	0.425 (0.65)
2	<i>B. cereus</i> (CLB2D)	0.175 (0.41)	0.285 (0.53)	0.420 (0.64)	0.746 (0.86)	0.483 (0.69)	0.418 (0.64)
3	<i>B. subtilis</i> (TSB5)	0.170 (0.41)	0.270 (0.51)	0.390 (0.62)	0.700 (0.83)	0.451 (0.67)	0.398 (0.63)
4	<i>B. cereus</i> (TSB4D)	0.165 (0.40)	0.235 (0.48)	0.362 (0.60)	0.567 (0.75)	0.415 (0.64)	0.325 (0.57)
5	<i>B. licheniformis</i> (TSB3)	0.160 (0.40)	0.197 (0.44)	0.320 (0.56)	0.478 (0.55)	0.360 (0.60)	0.310 (0.55)
6	Healthy control	0.150 (0.39)	0.167 (0.43)	0.198 (0.55)	0.306 (0.58)	0.234 (0.50)	0.199 (0.45)
7	Control	0.154 (0.38)	0.187 (0.40)	0.305 (0.44)	0.337 (0.53)	0.255 (0.48)	0.205 (0.44)
	SEd	0.08	0.15	0.29	0.50	0.74	0.97
	CD (P=0.05)	0.17	0.28	0.41	0.58	0.98	1.13

Note: Figures in parentheses are square root transformed values.

\* All the values are pooled data of two identical experiments.

**Plate.1** Confirmation of induction of defence enzymatic activity of peroxidase in tomato due to different isolates of *Bacillus* spp. under pot culture conditions using Native PAGE



1.	<i>B.weihenstephanensis</i> (TSB4) @ 5g / plant
2.	<i>B.cereus</i> (CLB2D) @ 5g / plant
3.	<i>B. subtilis</i> (TSB5) @ 5g / plant
4.	<i>B. cereus</i> (TSB4D) @ 5g / plant
5.	<i>B. licheniformis</i> (TSB3) @ 5 g / plant
6.	Inoculated control
7.	Healthy control

**Table.6** Influence of *B. weihenstephanensis* (TSB4) on total phenol, peroxidase, PPO and PAL activities in tomato under field conditions\*

Treatments	Total phenol (mg g <sup>-1</sup> root)	Peroxidase (min <sup>-1</sup> g <sup>-1</sup> root)	PPO (µg g <sup>-1</sup> )	PAL (µg g <sup>-1</sup> )
<i>B. weihenstephanensis</i> @ 1 kg /ha	120.66 (10.98)	0.850 (0.92)	0.260 (0.50)	0.189 (0.189)
<i>B. weihenstephanensis</i> @ 2 kg /ha	165.49 (12.86)	1.027 (1.01)	0.543 (0.73)	0.256 (0.50)
<i>B. weihenstephanensis</i> @ 3 kg /ha	228.79 (15.12)	1.179 (1.08)	1.795 (1.33)	0.397 (0.63)
<i>B. weihenstephanensis</i> @ 4 kg /ha	255.80 (15.99)	1.235 (1.11)	1.840 (1.35)	0.548 (0.74)
<i>B. weihenstephanensis</i> @ 5 kg /ha	287.99 (16.97)	1.349 (1.16)	1.520 (1.23)	0.632 (0.79)
Carbofuran3G @1kg a.i/ha	106.75 (10.33)	0.742 (0.86)	1.220 (1.10)	0.125 (0.35)
Untreated Control	68.50 (8.27)	0.500 (0.70)	0.138 (0.37)	0.154 (0.39)
SEd	0.84	0.09	0.05	0.03
CD(P=0.05)	1.39	0.32	0.25	0.18

Note: Figures in parentheses are square root transformed values.

\*All the values are pooled data from two sets of experiments.

Similar findings made earlier by Selvaraj and Ambalavanan (2013) in the plants of anthurium pretreated with BsW2 strain of *B. subtilis* and challenged with the pathogen *C. gloeosporioides* confirmed the enhancement of PPO activity following the application of *B. weihenstephanensis* (TSB4) in tomato. In contradictory the expression of various isoforms of PPO through native gel electrophoresis was observed by Nagendran *et al.*, (2013) in the application of *B. subtilis* (FZB24) upon challenge of *R. solani* in rice.

#### **Confirmation of presence of proteins in the isolates of *Bacillus* spp. through SDS – PAGE**

The analysis of SDS – PAGE for the presence of proteins in root knot nematode inoculated plants bacterized with the most effective five isolates of *Bacillus* spp. revealed the marked expression of proteins with molecular weight ranging from 14-95 KDa compared to

unbacterized plants (66 and 95 KDa). Among the five isolates of *Bacillus* spp., the *B. weihenstephanensis* (TSB4) displayed five numbers of bands with molecular weight ranging from 25 – 95 KDa compared to three numbers of bands displayed by *B. cereus* (CLB2D) and *B. subtilis* (TSB5) and two numbers of bands displayed by the remaining isolates.

It is observed that the defence enzymatic activity of total phenol, peroxidase, polyphenoloxidase and phenylalaninelyase enhanced in tomato treated with all the isolates of *Bacillus* spp. as curative measure to contain the incidence of root knot nematodes.

Among them the most effective endophyte of *B. weihenstephanensis* (TSB4) was found to be highly effective under field conditions also in the change of the defence enzymatic activity owing to *B. weihenstephanensis*

(TSB4) is presumed as biochemical mechanism of the bacterium. As above several authors demonstrated the increase in the defence enzymatic activity in general following the different methods of bacterization in many crops.

Hence the present findings fall in line with the report of Abbasi *et al.*, (2014) and Akram *et al.*, (2013) regarding the total phenol content. Similarly the present finding in respect of other defence enzymatic activity of peroxidase and polyphenoloxidase (Marait, 1973) and phenylalaninelyase (Akram *et al.*, 2013) is in accordance with earlier reports.

## References

- Abbasi, M.W., N. Ahmed, M. J. Zaki, S. Shahid Shuakat and D. Khan.2014.Potential of *Bacillus* species against *Meloidogyne javanica* parasitizing eggplant (*Solanum melongena* L.) and induced biochemical changes. *Plant Soil.*, 375:159-173.
- Akram, W., T. Anjum, B. Ali and A. Ahmad. 2013. Screening of native *Bacillus* strains to induce systemic resistance in tomato plants against *Fusarium* wilt in split root system and its field Applications. *International Journal of Agriculture and Biology.*, 15(6):1289–1294.
- Bloemberg, G.V., A.H.M. Wijfjes, G.E.M. Lamers, N. Stuurman and B.J.J. Lugtenberg. 2000. Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing three different auto-fluorescent proteins in the rhizosphere: new perspectives for studying microbial communities. *Mol. Plant-Microbe Interact.* 13:1170–76.
- Bottini, R., F. Cassan and P. Picolli. 2004. Gibberellin production by bacteria and its involvement in plant growth promotion. *Appl. Microbiol. Biotech.*, 65: 497–503.
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry.*, 72: 248-254.
- Bradley, D. J., P. Kjellborn and C. Lamb.1992. Elicitor and wound induced oxidative crosslinking of a plant cell wall proline-rich protein: A novel, rapid defense response. *Cell.*, 70: 21-30.
- Brueske, C.H. and V.H. Dropkin.1972. Free phenols and root necrosis in nematode infected tomato with root knot nematode. *Phytopathology.*, 63:329-334.
- Bryant, S.D. and E.L. Forrest.1979. Indole-3-acetic acid oxidase from Peas.I: Occurrence and distribution of peroxidative and non-peroxidative forms. *Plant Physiol.* 63: 696 - 699.
- Dickerson D. P., S. F. Pascholati, A. E. Hagerman, L. G. Butler and R. L. Nicholson. 1984. Phenylalanine ammonia-lyase and hydroxyl cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. *Physiol. Plant Pathol.*, 25:111–123.
- Doley, K. and P. Kaur. 2013. Disease management and biochemical changes in groundnut inoculated with *Glomus fasciculatum* and pathogenic *Macrophomina phaseolina* (tassi) Goid. *Plant Sciences Feed.*, 3(2): 21-26.
- Ganguly, A. K. and D. R. Dasgupta.1979. Investigations on peroxidase (E.C. 1.11.1.7.) and IAA oxidase from resistant and susceptible varieties of *Lycopersicon esculentum* infested with root-knot nematode, *Meloidogyne incognita*. *Ind. J. Nematol.*, 9: 60
- Gordons. A. and G. Paley. 1957. Formation of auxin from tryptophan through action of polyphenols. *Plant Physiol.*, 36: 838-845.
- Gutierrez-Manero, F.J., B. Ramos Solano, A. Probanza, J. Mehouchi, F.R. Tadeo and M. Talon. 2001. The plant growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiol Plantarum.*, 111:206 - 211.
- Hammerschmidt R, E.M. Nuckels and J. Kue.



1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber in *Colletotrichum lagenarium*. *Physiol. Mol. Plant Pathol.* 20:73–82.
- Hartree, E. F. 1955. Catalase, peroxidase and metmyoglobin as catalysts of coupled peroxidatic reactions. *Biochem. J.*, 60: 310-325.
- Ibrahim, S.K., 1991. Peroxidase isoenzymes from *Meloidogyne* spp. cultured on different hosts. *Revue de Nematologie.*, 14:335-344.
- Idriss E.E.S., O. Makarewicz, A. Farouk, K. Rosner, R. Greiner and H. Bochow. 2007. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB 45 contributes to its plant-growth-promoting effect. *Microbiology*, 148:2097–2109.
- Jayaraman, P.S., T.C. Peakman, S.J. Busby, R.V. Quincy and J.A. Cole 1987. Location and sequence of the promoter of the gene for the NADH-dependent nitrite reductase of *Escherichia coli* and its regulation by oxygen, the Fnr protein and nitrite. *J.Mol.Biol.*, 196: 781-788.
- Kumar A, S. Saini, A. Prakash and B.N. Johri. 2011. Influence of cultivation practices on phenotypic and genotypic diversity of antagonistic rhizobacteria isolated from soybean (*Glycine max* L.). In:1st Asian PGPR Congress for Sustainable Agriculture, Hyderabad. P.118. (Abs).
- Laemmli, U.K. and M. Favre.1973. Maturation of the head of bacteriophageT4.I DNA packaging events. *J. Mol. Biol.*, 80(4): 575-599.
- Lwin, M.L., M.M. Myint, T. Tar and W.Z.M. Aung.2012. Isolation of plant hormone (Indole - 3- acetic acid) producing rhizobacteria and study on their effects on maize seedlings. *Engineering Journal.*, 16(5): 152-158
- Malick, C. P. and Singh, M. B. 1980. Plant enzymology and histoenzymology. Kalyani Publications. New Delhi. 286 p.
- Maraite, H. 1973. Changes in polyphenoloxidases and peroxidases in muskmelon (*Cucumis melo* L.) infected by *Fusarium oxysporum* f. sp. *melonis*. *Physiol. Pl. Pathol.*, 3: 29-49.
- Mohanty, K.C., A.L. Ganguly and D.R. Dasgupta. 1986. Development of peroxidase (E.C.1.11.1.7.) activities in susceptible and resistant cultivars of cowpea inoculated with root knot nematode *Meloidogyne incognita*. *Indian J. Nematol.*, 16: 252-256.
- Nadolny, L. and Sequeira, L. 1980. Increases in peroxidase activities are not directly involved in induced resistance in tobacco. *Physiological and Molecular Plant Pathology.*, 16:1-8.
- Nagendran, K., G. Karthikeyan, P. Mohammed Faisal, P. Kalaiselvi, M. Raveendran, K. Prabakar and T. Raguchander.2013. Exploiting endophytic bacteria for the management of sheath blight disease in rice. *Biological Agriculture and Horticulture.*
- Patidar, R.K., D. Sen, K.M. Singh and R.C. Shakywar. 2013. Biotechnological tools for conservation of bioresources, *International Journal of Agriculture, Environment and Biotechnology.*, 6(2): 223-232.
- Pitcher, D. G., N. A. Saunders and R. J. Owen.1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett. Appl. Microbiol.*, 8:151–156.
- Ramamoorthy,V., R.Viswanathan, T. Raguchander, V. Prakasam and R. Samiyappan. 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protection*, 20:1-11.
- Ramanujam, B., H. Basha, V. Hemannavar, P. Chowdappa and R. Rangeshwaran. 2012. Induction of defense related enzymes and phenols in chilli plants by *Bacillus subtilis* against anthracnose pathogen, *Colletotrichum capsici*. *Indian Phytopath.*, 65(4): 382- 385.
- Richardson, A.E., J.M. Barea, A.M. McNeill and C. Prigent Combaret.2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth

- promotion by microorganisms. *Plant Soil*, 321:305–339.
- Selvaraj,T. and S.Ambalavanan. 2013.Induction of defense-related enzymes in anthurium by application of fungal and bacterial biocontrol agents against *Colletotrichum gloeosporioides*. *Int.J.Curr.Microbiol.App.Sci*.2(12): 661-670.
- Shadia, M. A. A. El., W. Nagdi and M. E. Moharam. 2013. Efficiency of the novel strain *Bacillus alvei* NRC -14 for biocontrol of parasitic nematode. *J. Agric. Food. Tech.*, 3(12): 31-40.
- Shukla, Y.M. and M.K. Chakraborty.1988.Biochemical studies on response of tobacco and tomato plants to root-knot nematode infection.*Tobacco Res.*, 14(1): 43-50.
- Singh C. 1983. Field Pea (*Pisum* spp.) In: Modern techniques of raising field crops. Singh C, (Ed). New Delhi: Oxford and IBH Publ. Co. Pvt. Ltd; Pp. 219–228.
- Tamalika Sarangi., 2014. Utilization of antinemic or antimicrobial peptide genes associated with *Bacillus* spp. in the management of root knot nematode *Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949 on tomato (*Solanum lycopersicum* Mill). Ph.D dissertation, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. P.371
- Tayal, M.S. and M.L. Agarwal.1982. Biochemical alterations in galls induced by *Meloidogyne incognita*. Some hydrolysing enzymes and related chemical metabolites. *Indian J. Nematol.*, 12: 379 - 382.
- Troll, J. and R.A. Rhode.1966. Pathogenicity of *Pratylenchus penetrans* and *Tylenchorhynchus claytoni* on turf grass. *Phytopathology*, 56: 995-998.
- Whipps, J.M. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.*, 52:487–512.

#### **How to cite this article:**

Tamalika Sarangi, S. Ramakrishnan and Nakkeeran, S. 2017. Ability of Antinemic/Antifungal *Bacillus* spp. for the Production of Plant Growth Hormones and Triggering Defense Enzymatic Activities. *Int.J.Curr.Microbiol.App.Sci*. 6(6): 2409-2423.  
doi: <https://doi.org/10.20546/ijcmas.2017.606.286>