ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 3, March 2016

Action of *Bacillus SPP* Endophytes of *Chenopodium Ambrosioides L* on *the Fusarium solani, Botrytis cinerea* and *Aspergillus niger*

Elhabchi Souaad[1], Elhartiti Abla[1], Hichar Abdelhadi[1], Bazdi Omar[1] and Ounine Khadija[2]

Research Scholar, Laboratory of biology and Health, Applied Microbiology Team; Faculty of Sciences Ibn Tofail University B.P: 133 14000, Kénitra-Morocco[1]

Professor, Laboratory of biology and Health, Applied Microbiology Team; Faculty of Sciences Ibn Tofail University B.P: 133 14000, Kénitra-Morocco[2]

ABSTRACT*:* Thirty one bacterial strains were isolated from root tissues, stems and leaves of Chenopodium ambrosioides L. The antifungal activity of these bacterial strains were tested in vitro by the technique of dual culture against *Botrytis cinerea* fungi; agent of gray rot on grapes, *Fusarium solani*; causal agent of *Fusarium wilt* of pepper and *Aspergillus niger*; causative agent of crown rot of peanuts. Four bacterial strains BR1, GT1, GR1 and GR2 induced inhibition of mycelial growth of *Botrytis cinerea, Fusarium solani* and *Aspergillus niger* with a rate greater than or equal 52%. These four strains are Gram positive *bacilli,* aerobic strict and capable of forming endospores. They belong to the family of *Bacillaceae.*

KEYWORDS: Fusarium solani, Botrytis cinerea, Aspergillus Niger, Chenopodium ambrosioides l, antifungal activity, Bacillaceae.

I. INTRODUCTION

In agriculture, fungal diseases are one of the constraints which are responsible for the loss of crop yields before and after harvest. Indeed 50% of fungal damage occurs in developing countries [1]. Among these diseases, there is *Fusarium wilt* of pepper which is caused by the phytopathogenic fungus *Fusarium solani* [2], the gray mold of grapevine which is caused by *Botrytis cinerea* [3] and the crown rot of peanuts which is caused by *Aspergillus niger* [4].

In order to combat these diseases, the chemical control is currently considered to be the most effective weapon as long as synthetic pesticides which are used have a negative impact on both the environment (accumulation of waste, soil pollution and ecological imbalance) on human health [5]. The biological control of phytopathogens is more beneficial to the environment when compared with the chemical control [6].

Several micro-organisms may be an alternative to this chemical control. Some have either the ability to inhibit different pathogens [7], or the ability to increase the defense mechanism of the plant [8].

The microorganisms of the rhizosphere to the origin of this inhibition use more than one mechanism for the elimination of pathogens and the reduction of the disease frequency [9]. They act in part by secreting antimicrobial substances which have either an antifungal activity [10] or an antibacterial activity [11].

Several species of the genus Bacillus are effective in the biocontrol of various plant pathogenic fungi [12]. They have the ability to reduce plant diseases caused by Oomycetes [13] through the production of secondary metabolites with antifungal and antibacterial properties such as fengycin, bacillomycin D, zwittermicin A, the iturin A, and the surfactin. The antibiotic-producing bacteria are *B. subtilis, B. mycoides, B. amyloliquefaciens, B.polymexa, B.licheniformis, B.circulans and B.cereus.* [14, 15, 16, 17, 18]. The objective of this study is the isolation and the identification of endophytic bacteria having an antifungal effect on the three phytopathogenic fungi (*Aspergillus niger*, *Fusarium solani and Botrytis cinerea)* from different tissues of *Chenopodium ambrosioides L.*

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 3, March 2016

II. MATERIALS AND METHODS

1. Vegetal material

The plants of *Chenopodium ambrosioides L* were collected from the region of Gharb-chrarda-beni hssen, Morocco. Leaves, stems and roots were washed with sterile distilled water and then superficially disinfected with ethanol at 70% and sodium hypochlorite (NaClO) at 3% and then washed several times with sterile distilled water.

2. Isolation of endophytic bacteria of *chenopodium ambrosioides l*

Each tissue (leaves, stems or roots) was added with 0.9% NaCl (1:10) and macerated with mortar and pestle. One gram of macerated tissue was placed in a tube containing 9 ml sterile 0.9% NaCl. One milliliter of appropriate (10^{-2} *to* 10^{-7}) dilution of tissue was plated on Petri dishes containing nutrient agar medium (GN) for the total flora. The Petri dishes were incubated at 28˚C for 24 to 48 hours. All colonies observed are collected, purified, stored at -4 ° C in slant tubes containing nutrient agar after growth at 28 ° C and in 50% glycerol at -20 ° C.

3. Antagonist power of bacterial isolates of *Chenopodium ambrosioides l* in vitro

The antagonist power of isolates is studied in vitro vis-à-vis the three fungi *Aspergillus niger, Fusarium solani and Botrytis cinerea* by the dual culture technique [19].

The tests of fungal antagonism were made on a potato dextrose agar medium (PDA). Bacterial isolates were cultured in nutrient broth for 24 hours at 28 C. An aliquot of 10µl of the bacterial suspension was inoculated as two streaks at the edge of the Petri plate, while a fungal disc of 7 mm from a culture of 7 days is placed in the center of the PDA plate. For the control, a mycelial disk of the pathogen was placed in the center of the PDA plate. After 7 days of incubation at 28 ° C in the dark, the inhibition of fungal radial growth by the antagonist is calculated using the following formula [20]:

$$(\%)\ \text{Inhibition} = [r1-r2/r1] \times 100$$

r1: radial growth of the mycelium witness.
r2: The radial mycelial growth in the presence of the antagonist.

4. Identification

The isolated bacterial strains are subject to morphological and biochemical identification tests according to the Manual of Bergey's Determinative Bacteriology, 2001.

III.RESULTS

1. isolation of endophytic bacteria from *Chenopodium ambrosioides l*

We isolated and purified 31 bacterial strains from plant tissues of *Chenopodium ambrosioides L*, including 10 strains which are obtained from the roots, 13 strains from the stems and 8 from the leaves.

2. antagonist power of bacterial isolates of *Chenopodium ambrosioides L* in vitro:

According to the resultants shown in table 1, figure 1, figure 2 und figure 3. We notice that among the 31 isolated bacterial strains, four bacterial isolates inhibited the growth of three phytopathogenic namely fungi *Botrytis cinerea*, *Fusarium solani* and *Aspergillus niger*.

The BR1, GT1, GR1 and GR2 strains showed remarkable efficiency and they inhibited the mycelial growth of *Botrytis cinerea* and *Aspergillus niger* with a higher percentage of inhibitions or equal to 61.36%. While these isolates reduced the mycelial growth of *Fusarium solani* with an inhibition rate above 52%.

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 3, March 2016

ISOLATES	inhibition rate of mycelial growth		
	Aspergillus niger	*Botrytis cinerea*	*Fusarium solani*
witness	0٪	0٪	0٪
GT1	71.60٪	75٪	52٪
GR1	70.45٪	71.15٪	52٪
GR2	64.77٪	75٪	52.38٪
BR1	61.36٪	61.53٪	54٪

Table 1: inhibition rate of mycelial growth by bacteria isolated from *Chenopodium ambrosioides L.*

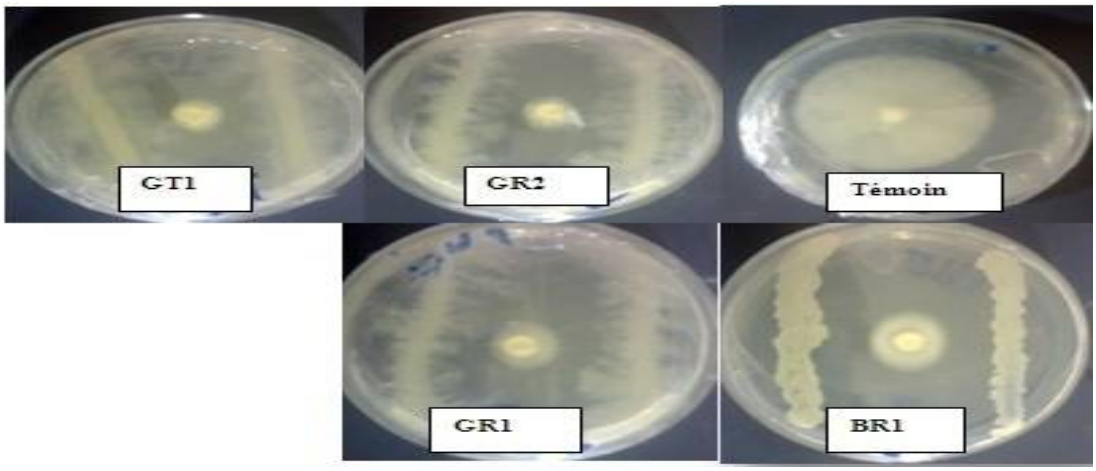

Figure1: Effect of the bacterial strains BR1, GT1, GR1 and GR2 on the mycelial growth *of Botrytis cinerea.*

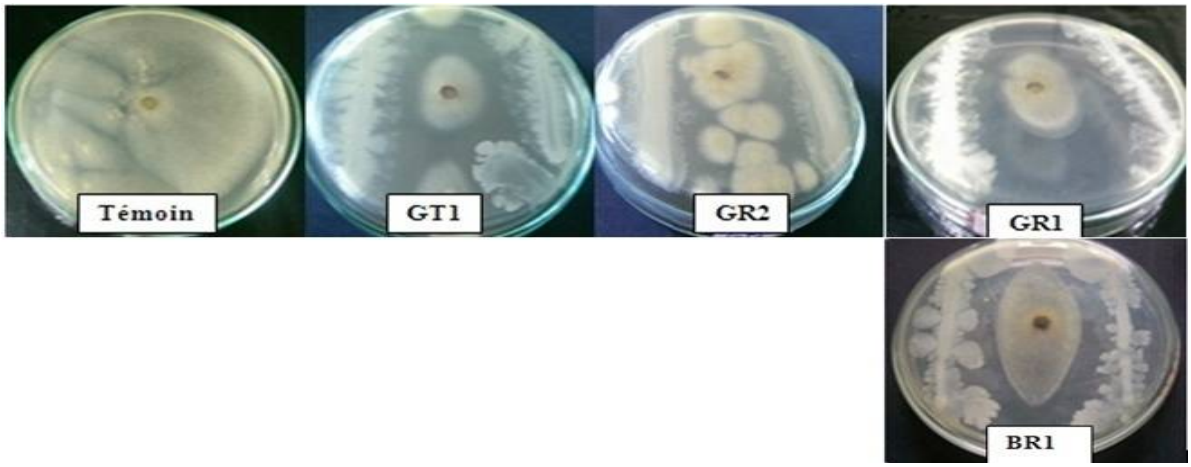

Figure 2: Effect of the bacterial strains GT1, GR2, GR1 and BR1 on the mycelial growth of *Aspergillus niger.*

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 3, March 2016

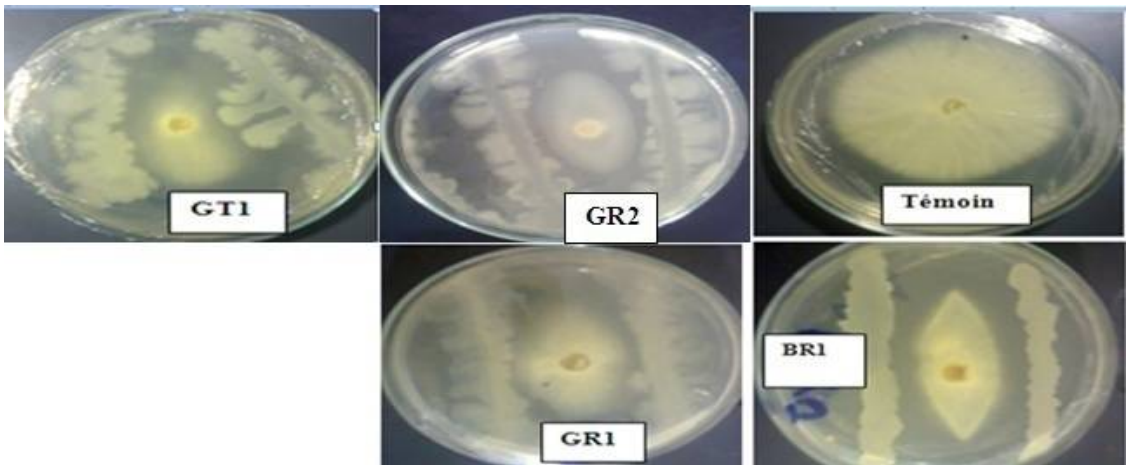

Figure 3: Effects of the bacterial strains BR1, GT1, GR1 and GR2 on the mycelial growth of *Fusarium solani.*

3. IDENTIFICATIONS

The biochemical identification of the four strains BR1, GT1, GR1 and GR2 having presented an antagonistic power towards three fungi is presented in Table 2. These isolates are Gram positive bacilli, mobile capable of forming endospores. They strictly have an aerobic metabolism and positive catalase. They are capable of hydrolysing casein, gelatine and starch and reducing nitrate to nitrite or ammonia, they are devoid of deaminase tryptophan and tryptophanase.

The BR1 strain is Voges-Proskauer (VP) and citrate positive while GT1 GR1 and GR2 are Voges-Proskauer (VP) and citrate negative. According to Bergy's manual of determinative bacteriology 2001, these strains belong to the Bacillus spp genus.

	the isolates			
The tests	**BR1**	**GR1**	**GR2**	**GT1**
Oxidase	-	+	+	+
Methyl red	-	+	+	+
Voges- Proskauer	+	-	-	-
Citrate	+	-	-	-
Mannitol's fermentation	-	+	+	+
Urease	-	+	+	+

Table 2: the biochemical tests of the bacterial isolates of *Chenopodium ambrosioides L.*

IV. DISCUSSION

The antagonistic effect of *Bacilli spp* BR1, GR1, GR2 and GT1 isolated from *Chenopodium ambrosioides L* in vitro vis-a-vis the three fungi *Fusarium solani, Botrytis cinerea* and *Aspergillus niger* was observed. These isolates reduced the mycelial growth of phytopathogenic fungi *Fusarium solani* with an inhibition's rate greater or equal to 52% while they have shown their antagonistic effectiveness in inhibiting more than 61.36% of mycelial growth of *Aspergillus niger* and *Botrytis cinerea.*

Several bacterial strains of the *Bacillus* genus isolated from various habitats have significant efficacy in biological control against pathogenic fungi [22]. Also Asaka and Shoda. [23]; Emmert and Handelsman. [24] reported that the bacterium *Bacillus subtilis* rhizosphere plays a very important role in the suppression of phytopathogenic agents. Trotel

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 3, March 2016

Aziz et al. [25] demonstrated that the *Bacillus subtilis* strain is capable of inhibiting the growth of *Botrytis cinerea* by the dual confrontation technique.

Other studies have shown that the *Bacillus spp* have strong a antifungal activity in vitro vis-à-vis *Aspergillus niger* [26, 27]. In a previous report, Eshetu Belete et al. [28] declared that some *Bacillus spp* reduced the mycelial growth of the pathogen agent *Fusarium solani.*

In this study, the bacillus spp BR1, GR1, GR2 and GT1 inhibited the mycelial growth of *Fusarium solani*, *Botrytis cinerea* and *Aspergillus niger* with a different percentage of inhibition. This suggests that the type of secondary metabolite, which is produced by these isolates, varies [29]. The Bacillus spp are known for the production of different types of antibiotics that are used in the biological fight against phytopathogens [30]. Thus, the production of antibiotics by Bacillus spp and their uses in the biological control of phytopathogens has also been reported by Killani et al. [31]. In another earlier study by Young et al. [32] stated that the bacterium *B. subtilis* produces at least five different antibiotics, namely subtillin, bacitracin, bacillin, subtenolin and bacillomycin. Other antibiotics produced by Bacillus spp are fengycin A and fengycin B, mycobacillin, iturin A, surfactin, mycosubtilin, fungistatin, subsporin, bacilysin, chlorotetaine [30].

V.CONCLUSION

The isolates of *Bacillus spp* BR1, GT1, GR1 and GR2 showed significant antagonistic activity in vitro on the mycelial growth of *Fusarium solani*, *Aspergillus niger* and *Botrytis cinerea*. These results show that these bacterial strains can be an effective biocontrol against phytopathogenic fungi and can limit the use of the chemical inputs.

REFERENCES

[1] Wojcieh, J. J., and Lise, K., "Biological control of postharvest diseases of fruits", Annu. Rev. Phytopatol. 40:411-441, 2002.
[2] Sundaramoorthy, S., Raguchander, T., Ragupathi, N., and Samiyappan, R., "Combinatorial effect of endophytic and plant growth promoting rhizobacteria against wilt disease of Capsicum annum L. caused by Fusarium solani", Biological Control 60 (2012) 59–67, 2011.
[3] Elad, Y., Williamson, B., Tudzynski, P., and Delen, N., **"***Botrytis* spp. and diseases they cause in agricultural systems-an introduction. In: Botrytis: Biology, Pathology and Control", Kluwer Academic Publishers, Dordrecht, the Netherlands, pp. 1-9, 2004.
[4] Magnoli, C., Astoreca, A., Ponsone, L., Fernández-Juri, M.G., Chiacchiera, S., and Dalcero, A., "Ochratoxin A and the occurrence of ochratoxin A-producing black *aspergilli* in stored peanut seeds from Córdoba, Argentina", Journal of the Science of Food and Agriculture 86, 2369–2373. 2006.
[5] Thakore, Y., "The biopesticides market for global agricultural use", Industrial Biotechnology. 2(3):294-208, 2006.
[6] Nautiyal, C.S, "Biocontrol of plant diseases for agricultural sustainability". In: Upadhyay, R.K, Mukerji, K.G., Chamola, B.P. "Biocontrol Potential and its Exploitation in Sustainable Agriculture", vol.l: Crop Diseases, Weeds, and Nematodes. Ed. Kluwer Academic, New York, pp.9-23, 2000.
[7] McSpadden Gardener, B.B. and Fravel, D.R, "Biological control of plant pathogens: Research, commercialization, and application in the USA", Online. Plant Health Progress doi:10.1094/PHP-2002-0510-01-RV, 2002.
[8] Silva, H.S.A., Romeiro, R.S., Carrer-Filho, R., Pereira, J.L.A., Mizubuti, E.S.G. and Mounteer, A, "Induction of systemic resistance by *bacillus cereus* against tomato foliar diseases under field conditions", Journal of phytopatology, 152, pp.371-375, 2004.
[9] Chet, I., Ordentlich, A., Shapira, R., and Oppenheim, A., "Mechanisms of biocontrol of soil-borne plant pathogens by Rhizobacteria", *Plant and Soil*, 129: 85-92, 1990.
[10] Chen, X., Koumoutsi, A., Scholz, R., Eisenreich, A., Schneider, K., Heinemeyer, I., Morgenstern, B., Voss, B., Hess, W., Reva, O., Junge, H., Voigt, B., Jungblut, P., Vater, J., Sussmuth, R., Liesegang, H., Strittmatter, A., Gottschalk, G., and Borriss, R., "Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42", *Nature biotechnology* 2007, 25:1007-1014, 2007.
[11] Chen, X., Koumoutsi, A., Scholz, R., Schneider, K., Vater, J., Sussmuth, R., Piel, J., and Borriss, R., "Genome analysis of *Bacillus amyloliquefaciens* FZB42 reveals its potential for biocontrol of plant pathogens"**,** *Journal of biotechnology* 2009, 140**:**27-37, 2009.
[12] Swain, M.R., and Ray, R.C., "Biocontrol and other beneficial activities of bacillus subtilis isolated from cowdung microflora". Microbiological Research, doi: 10. 1016/j. micres. 2006.10.009, 2006.
[13] Handelsman, J., and Stabb, E.V., "Biocontrol of soilborne plant pathogens", the plant cell. 8, pp. 18855-1869, 1996.
[14] Athukorala, S.N.P., Fernando W.G.D., and Rashid, K.Y., "Identification of antifungal antibiotics of *Bacillus* species isolated from different microhabitats using polymerase chain reaction and MALDI-TOF mass nul spectrometry", Canadia Jorna of Microbiology 55:1021-1032, 2009.
[15] Ramarathnam, R., Bo S., Chen Y., Fernando W.G.D., Gao X., and de Kievit T., "Molecular and biochemical detection of fengycin- and bacillomycin D-producing *Bacillus* spp., antagonistic to fungal pathogens of canola and wheat", Can. J. Microbiology.53:901 -911, 2007.
[16] Milner, J.L., Stohl, E.A., and Handelsman, J., "Zwittermicin A resistance gene from *Bacillus cereus"*, J. Bacteriol., 178, pp. 4266-4272, 1996a.
[17] Drablos, F., Nicholson, D., Ronning, M., "EXAFS study of zinc coordination in bacitracin A", Biochimica et Biophysica Acta 1431 (1999) 433-442, 1999.
[18] Perez, P.M., Belanger, R.R., Paulitz, T.C., Benhamou, N., **"**Antimicrobial activity determined in strains of *Bacillus circulans* cluster", Folia Microbiol, 38, pp. 25-28, 1993.

ISSN(Online): 2319-8753
ISSN (Print): 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 3, March 2016

[19] Berg, G., Krechel, A., Ditz, M., Sikora, RA., Ulrich, A., and Hallmann, A., "Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi", FEMS Microbiol Ecol, 51:215–29, 2005.
[20] Trivedi, P., Pandey, A., and Palni, L.M.S., "In vitro evaluation of antagonistic properties of *Pseudomonas corrugates"*, *Microbiol. Res*. 163: 329-336, 2008.
[21] Bergey´s Manual of Systematic Bacteriology, 2001, seconded**.**
[22] Bargabus, R.L., Zidack, N.K., Sherwood, J.E., and Jacobsen, B.J., "Oxidative burst elicited by Bacillus mycoides isolate Bac J, a biological control agent, occurs independently of hypersensitive cell death in sugar beet", Mol. Plant Microbe Interact. 16, 1145–1153, 2003.
[23] Asaka, O. and Shoda, M., "Biocontrol of *Rhizoctonia solani* damping off of tomato with *Bacillus subtilis* RB14", Appl. Environ. Microbiol. 62, 4081–4085, 1996.
[24] Emmert, E.A.B. and Handelsman, J., "Biocontrol of plant disease: a (Gram-) positive perspective". FEMS Microbiol. Lett. 171, 1–9, 1999.
[25] Trotel-Aziz, P., Couderchet, M., Biagianti, S., and Aziz, A., "Characterization of new bacterial biocontrol agents *Acinetobacter*, *Bacillus*, *Pantoea* and *Pseudomonas* spp. Mediating grapevine resistance against *Botrytis cinerea"*, Environmental and Experimental Botany 64 (2008) 21–32, 2008.
[26] Watcharin, Y., Pruksa, L., Sopone, W., Neung, T., Nantakorn, B., Nobuhiko, N., and Panlada, T., "Improvement of peanut rhizobial inoculant by incorporation of plant growth promoting rhizobacteria (PGPR) as biocontrol against the seed borne fungus, *Aspergillus niger"*. Biological Control 63 (2012) 87–97, 2012.
[27] Podile, A.R., and Prakash, A.P., "Lysis and biological control of Aspergillus niger by Bacillus subtilis AF 1", Canadian Journal of Microbiology 42, 533–538, 1996.
[28] Eshetu belete, A.A., and Seid, A., "Antagonistic effect of native Bacillus isolates against black root rot of faba bean", African Crop Science Journal, Vol. 23, No. 3, pp. 249 – 259, 2015.
[29] Ahmed-Idris, H., Labuschagnea, N., and Korstena, L., "Screening rhizobacteria for biological control of *Fusarium* root and crown rot of sorghum in Ethiopia", *Biological Control* 40: 97-106, 2007.
[30] Arguelles-Arias, A., Ongena, A., Halimi, A., Lara, Y., Brans, A., Joris, B., and Fickers, P., "*Bacillus amyloliquefaciens* GA1 as a source of potent antibiotics and other secondary metabolites for biocontrol of plant pathogens", Microbial Cell Factories 8: 1-12, 2009.
[31] Killani, A.S., Abaidoo, R.C., Akintokun, A.K., and Abiala, M.A., "Antagonistic effect of indigenous *Bacillus subtilis* on root-/soilborne fungal pathogens of cowpea". *Researcher* 20: 11-18, 2011.
[32] Young, F.E., Tupper, J., and Strominger, J.L., "Autolysis of cell walls of *Bacillus subtilis* mechanism and possible relationship to competence", *Journal of Biology and Chemistry* 249: 3600-02, 1974.