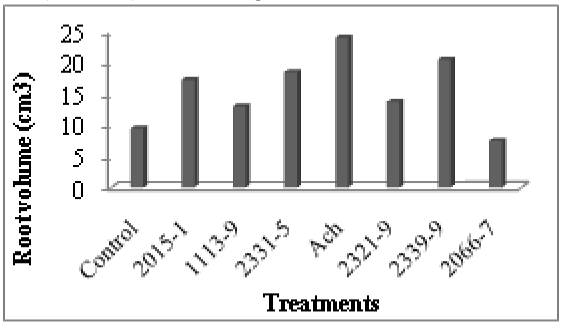
Table 2: Degree of significance of treatment compared to the control pathogen (strain P512C9)

Treatment / Parameter	2015-1 + Pathogen	1113-9 + Pathogen	2331-5 + Pathogen	Ach + Pathogen	2321-9 + Pathogen	2339-9 + Pathogen	2066-7 + Pathogen
Diameter of the stem	0.01	> 0.05	0.01	0.001	0.01	0.01	0.01
Length of the plant	0.04	0.01	0.02	0.01	0.04	0.01	> 0.05
Chlorophyll content	0.005	0.03	0.005	0.001	0.01	0.01	0.01
Leaf area	0.01	> 0.05	0.02	0.001	0.02	0.02	> 0.05
Average weight of tubers	> 0.05	> 0.05	> 0.05	0.02	> 0.05	> 0.05	> 0.05
Root volume	> 0.05	> 0.05	> 0.05	0.05	> 0.05	> 0.05	> 0.05
Root length	0.02	0.05	0.05	0.005	0.01	0.01	> 0.05
Caliber of the tuber	0.01	0.05	0.005	0.005	> 0.05	0.005	0.005
Aboveground biomass	> 0.05	> 0.05	> 0.05	0.05	> 0.05	> 0.05	> 0.05

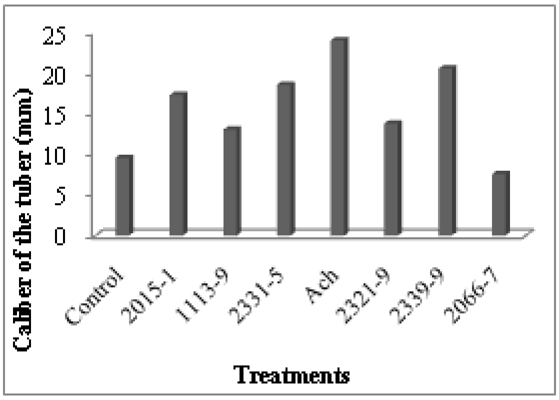
3.4 Effect of PGPR on the potato crop

In aim to evaluate the multiple activities of antagonistic agents to promote the potato plant growth, it was found necessary to control the parameters of the development and growth of the crop; thereby different parameters described previously were measured on the treatments with only the antagonistic strains during the crop cycle in October 2013 - February 2014.

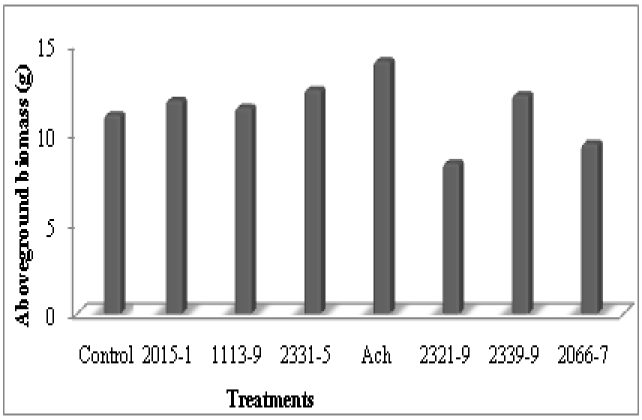
The data of the effect of PGPR strains showed slight differences with regard to the above ground biomass (Figure 5-c), the plant height, the foliar area, and the root length compared to sterile distilled water-treated controls. However, in treatment with strain Ach, the caliber of tuber (Fig 5-b), the root volume (Figure 5-a) and the chlorophyll content (Figure 5-d) weresignificantly higher than that of control. However, the statistical analysis doesn't showed significant results compared to negative control. Except, the treatment by antagonist strain Ach which showed a high degree of significance to the physiological parameters; chlorophyll content (P = 0.03), the caliber of tuber (P = 0.01) and the root volume (P= 0.05) than to negative control.



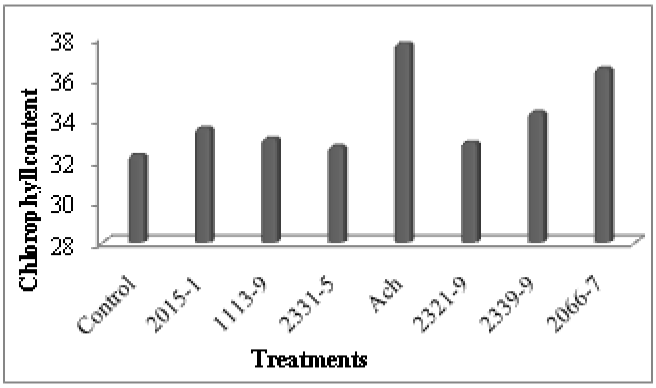
(a)



(b)



(c)



(e)

Figure 5: Effect of the agents benefic (PGPR) on chlorophyll content (d), the volume and length of the root (a-e), aboveground biomass (c) and caliber of tuber (b) evaluated during the cycle of potato crop.

4. Discussion

In Morocco, *P. carotovorum* is a well known pathogen of potato and was detected in many other Mediterranean countries [22]. To evaluate the activity of thirty antagonists (bacteria and fungus) against *P.carotovorum*, three experiments were carried out; the antibiosis test, slices of potato test and *in situ* greenhouse test. Results indicated that the investigated antagonists significantly reduced potato soft rot disease compared to untreated controls. However, among the antagonists, strain 2321-9 had a slight effect against *P. carotovorum*, the strains (2339-9, 2015-1, 2331-5, 1113-9 and 2066-7) presented an average effect. Furthermore the yeast-like Ach showed a high activity, and was the most effective and stable antagonist. This is consistent with data of a previous study in which strain Ach1-1 of same species, *Aureobasidium pullulans*, among different potential antagonistic microorganisms isolated from apple surfaces, has shown a protective level of more than 90% against several pathogens, such as; *P. expansum* [16]-[23]. Other strains of *A. pullulans* reported in other works also displayed high antagonistic activities not only against *P. expansum* on apples but also against major postharvest pathogens on several important crops [24]-[25]-[26]-[27]-[28]-[29]-[30].

On the other hand, the results of the present study demonstrated that the antagonistic yeast strain Ach can completely inhibit the development of soft rot disease *in vivo* and *in situ*, with regard to other antagonists (1113-9, 2015-1, 2066-7) that have significantly inhibited the growth of soft rot bacteria *in vitro* (test antibiosis) than the strain Ach. Thus, those data are consistent with several studies reporting the lack of correlation between *in vitro* antibiosis and bio-control. For example, [31] proved that no significant correlation was observed between the *in vitro* growth of *Phytophthora cactorum* in presence of a bacterium and the protection from infection of apple seedlings by that bacterium in the green-house [31]. Additionally, *in vitro* screening for antibiosis is frequently used to select prospective antagonists [32] which may be used for bio-control essays in greenhouse as well in field.

In addition, it is interesting to note that, some strains were isolated from the same fruit and have not prove the same capacity to reduce the soft rot disease. Such as, the strains 2330-3, 2328B-3, 2217-3a, 1113-9, Ach, all were isolated from apple fruit but the yeast-like *Aureobasidium pullulans* (Ach) has demonstrated a high degree of efficacy compared to other presumed antagonistic organisms. Consequently, the present work is in agreement with other previously published research that found those no relationship between the ecological origins where the antagonist was isolated and its effectiveness against diseases. Moreover, the efficacy in the reduction of soft rot produced by *P. carotovorum in vivo* and *in situ* has varied between the strains antagonists [33]-[34]-[35].

In the current work, the effect on the stimulation of the growth of the potato plant by the PGPRs strains was also evaluated. Results showed that the strains tested didn't have significant effects, in particularly *A. pullulans* strain Ach which produce significant differences in variation of chlorophyll content, root volume and caliber of the tubers indicating that the strain Ach had an important effect on the assimilation of nutritive element by plant. In conclusion, the present work has provided strong evidence from both *in vivo* and *in situ* of the bio-control activity of *A. pullulans* strain Ach against *P.carotovorum*. The observed reduction of soft rot by *A. pullulans* is suggesting a complex mode of action still poorly understood. The ability of the yeast antagonistic to outcompete the pathogen for nutrients and space can be one of the mechanisms in the bio-control as previously suggested in other studies. Therefore, further investigation will be carried out in this direction in order to (1) find the main mechanisms involved in the bio-control activity of *A. pullulans* strain Ach against *P. carotovorum*, (2) search for the formulation of *A.pullulans* as biofungicides to control of soft rot disease caused by *P. carotovorum*.

Table 3: list of the antagonistic strains selected, examined for biological control against soft rot of potato

Code of strain	Date of collection	Origin	Sampling location	specie
2015-1	24/11/2011	Compost	Meknès	*Bacillus cereus*
1113-9	15/02/2004	apple	Market	*A. pullulans*
Ach	15/02/2004	apple	Market	*A. pullulans*
2066-7	08/03/2012	olive tree	Taounate	*P. agglomerans*
2339-9	5/03/2013	Apple tree	El Hajeb	-
2321-9	20/12/2012	Olive tree	ketama	-
2331-5	12/02/2013	Apple tree	Meknès	-
Ach1-1	15/02/2004	Apple	Market	*A. pullulans*
2321-5	20/12/2012	Olive tree	Ketama	-
2027-2	3/02/2012	Olive tree	INRA Meknes	*Bacillus cereus*
2236-2	27/06/2012	Cognassier	BeniMellal	-
2321-10	20/12/2012	Olive tree	Ketama	-
2330-3	12/02/2013	Apple tree	Meknès	-
2322-3	20/12/2012	Olive tree	Ketama	-
2321-6	20/12/2012	Olive tree	Ketama	-
2216-11	15/05/2012	Cognassier	Bouderbala	-
2321-11	20/12/2012	Olive tree	Ketama	-
2077-5	03/04/2012	Olive tree	My Driss Zarhoun	-
2328B-3	31/12/2012	Apple tree	Fès	-
2217-3b	15/05/2012	Apple tree	Tifrit-Bouderbala	-
2217-3a	15/05/2012	Apple tree	Tifrit-Bouderbala	-
2332A-2	18/02/2013	Apple tree	Fès	-
2074-1TC	03/04/2012	Olive tree	My Driss Zarhoun	*P.agglomerans*
2330-4	12/02/2013	Apple tree	Meknès	-
2339-7	5/03/2013	Apple tree	El Hajeb	-
2328B-5	31/12/2012	Apple tree	Fès	-
2026-2	3/02/2012	Compost	INRA Meknes	-
2332A-4	18/02/2013	Apple tree	Fès	-
2320-4	12/12/2012	Apple tree	El Hajib	-

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Author Profile

Hind FAQUIHI: received his M.S degree in Bioengineering from Faculty of Science and Technology in 2011. She is currently PhD student in Molecular Microbiology and Biotechnology in the Department of Biology, Faculty of Science and Technology, University Hassan II, Mohammedia – Casablanca, Morocco in cooperation with Research Unit of Plant Protection, National Institute of Agronomic Research – Meknes, Morocco.