ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

Bioprocessing Of Brevibacillus Brevis and Bacillus Polymyxa: A Potential Biocontrol Agent of Gray Mould Disease of Strawberry Fruits

Wafaa M. Haggag , F. Abd-El-Kareem and S.D. Abou-Hussein

Abstract- Gray mold, caused by Botrytis cinerea is one of the important strawberry diseases that causes losses before or after harvest wherever strawberry. The effects of preharvest spray with promising bacteria i.e. Bacillus brevis and Bacillus polymyxa on gray mould and quality of strawberry fruits pre and postharvest were evaluated. In "in vitro" assays cells and their filtrates of both isolates strongly inhibited B. cinerea growth. Moreover, liquid chromatography–mass spectrometry analysis was carried out to identify the antifungal components of the most effective culture filtrates against gray mold pathogen. Bacillus polymyxa and B. brevis were found to produce large amounts of peptide polymxin B and gramicidin S, respectively. In order to standardize the mass and metabolite production some cultural conditions like different incubation time in hours, pH, carbon sources and concentrations and nitrogen source were determined. During fermentation, growth, pH and antibiotics production were monitored. Preharvest application of liquid formulation of culture filtrates of certain microbial isolates provided an effective control of fruit gray mould disease pre and protect fruit during storage than the tested fungicide at the recommended levels. These results suggested that integration of preharvest spray with biocontrol may be a promising management strategy for decay control and quality maintenance of strawberry.

Key words: **Strawberry fruits -Gray mold, - Botrytis cinerea.**

I. INTRODUCTION

Gray mould, caused by *Botrytis cinerea* Pers., Fr . is one of the important strawberry disease that causes losses before or after harvest wherever strawberry (*Fragaria ananassa* L.) (2; 16; 17) . Gray mould management is based principally on chemical control, but fungicide application may cause problems such as toxic residues on the fruits and selection of resistant isolates of the pathogen (8, 10, 13, 14). Additionally, fungicide application at flowering may reduce pollen viability and consequently hinder fruit formation (4) .Control of fungal pathogens is based on the use of agronomic practices and pesticides, but widespread application of chemicals inundates the agro-eco systems with toxic compounds that affect the balance of the natural food chain. The main problem regarding treatment in the post harvest period is the presence of toxic residues on fruit, due to the short time elapsing between treatment and consumption. This problem has led to the need to find new methods of controlling microbiological alterations by using the natural micro flora present on fruits which play an important role in the control of fungal pathogens. It is very important therefore to isolate and characterize microorganisms with antagonistic activity so that biological control can be put into practice (14). Most sustainable and environmentally acceptable control may be achieved using biocontrol agents (BCAs) (5; 6). The current situation is mainly focused on biological control (BCAs).Various microbial antagonists have been investigated as potential antifungal biocontrol agents of plant diseases. We isolated more some microorganisms from a variety of strawberry fruit included *bacteria* as Bacillus *brevis* and *Bacillus polymyxa*. *B. brevis* has been shown to produce a single cyclic as decapeptide antibiotic gramicidins S which has been shown to be fungicidal (7). The modes of action that play a role in disease suppression by these bacteria include antibiosis, production of antibiotic peptide (1) . Specific objectives of this work included: (a) to determine in vitro the antagonistic mechanism of the bacteria i.e. *Bacillus brevis* and *Bacillus polymyxa* against *Botrytis cinerea* the causal agent of grey mould , (b) to scale-up the submerged fermentation (c) to study the shelf life of liquid formulation containing oil and/or sugar, alginate, and (d) to determine the effect of foliar application of biocontrol agents pre harvest on the control of grey mould in vivo and during storage periods.

II. MATERIALS AND METHODS

A. Pathogen

Botrytis cinerea pers.; Fr. the causal agents of gray mold disease on strawberry fruits was kindly isolated from diseased strawberry fruits from a field at El Sharkia governorate and maintained on potato carrot agar. Fungal culture was identified in Plant Pathology Department, National Research Centre, and Egypt. For conidial production, *B. cinerea* were grown on PDA (Potato Dextrose Agar) at 20–25 °C. When the mycelium appeared, cultures were kept at 15 °C for inducing sporulation. After a week, spores were harvested and suspended in 10 ml of sterile distilled water containing 0.05% (v/v) Tween 80. The concentration of spore suspension was adjusted with sterile distilled water to 1×10^6 spores/ml.

B. Biocontrol agents (BCAs)

Bacillus brevis and *Bacillus polymyxa* previously isolated from strawberry plants were used for this work. These isolates were identified in Plant Pathology Department, National Research Centre based on a great variety of morphological, cultural, physiological and biochemical characteristics. These isolates were grown in nutrient broth at 26^0C for 24 h. Bacteria were spread onto

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

nutrient agar plates from stocks. These plates were incubated overnight at 25^0C and used to inoculate 100 ml nutrient broth (Oxoid) in 250 ml conical flasks . The cells were diluted to 2 x 10^5 cells/ml with sterile water and then resuspended in 0.01 M potassium phosphate buffer (pH 7.0) plus 0.01% Tween-80 as surfactant.

C. Biological evaluation

A streak assay was done on PDA; an agar disk from *B. cinera* was placed at the center of the dishes. BCAs were streaked at two sides at about 2 cm from the center. The plates were incubated at 28 for 48 h and evaluated for inhibition zones.

D. Production of antifungal compounds

A cell-free filtrate from BCAs was used for evaluating antifungal compounds production by these bioagents. The microorganisms were cultivated in 100 ml of YGM broth for 48 h at 28 °C. After the incubation period, cells were centrifuged at 2500 g for 15 min (Sorvall centrifuge), the supernatant fluid decanted and a 2 ml aliquot filtered through a sterile 0.2 µm Acrodisc filter (Gelman UK). The cell-free filtrates were then screened for antagonism against B. cinera, in Petri dishes on PDA. Petri dishes were seeded with 200 µl of a phytopathogen suspension containing 106 spores/ml. After the dishes were seeded, they were stored at 4 °C over-night and then, holes (diameter 10 mm) were made in the medium of each Petri dish with a sterile cork borer. Holes were filled to capacity (200 µl) with the cell-free filtrate, and then the plates were incubated at 28 °C for 48 h and evaluated for inhibition zones.

In order to evaluate antagonistic activity of BCAs against spore germination of *B. cinerea*, in vitro assays on special slides for micro culture were performed. In these assays was also determined the minimum filtrate concentration necessary for inhibiting the conidial germination of the phytopathogen. Slides were incubated in a wet chamber at 28 °C during 48 h. After this time, these preparations were observed with a light microscope (Olympia) at a magnification of ×500. Reduction of spore germination of pathogen was determined 72 h after their application.

E. Separation and purification of various fractions

The separation of various fractions was carried out by column chromatography, analyzed and purified by thin layer chromatography (TLC), until homogeneity of the fraction achieved. The homogeneity and the purity of the different fractions were checked by HPLC.

F. Optimization of incubation period, pH and carbon and nitrogen sources concentration for antibiotic peptides production

The optimization of composition of incubation period, and cultural conditions was carried out based on stepwise modification of the governing parameters for antifungal production. The cultures were transferred to seed broth (200 mL of Medium) contained in a 500 mL Erlenmeyer flask and incubated at 30°C on a rotary shaker (175 rpm) for 6-8 hours. A 500 mL Erlenmeyer flask containing 200 mL of the same seed medium was incubated as specified above. The seed culture was transferred to a 5 liter fermenter containing each one 3.5 liter of the three liquid media

G. Assay in liquid cultures

Bioagents growth was estimated directly by spectrophotometric measurement of the OD600 (Amax) using a PM 2A spectrophotometer and dry biomass concentration (bmax). Changing the pH 3 to 10 in the production medium the effect of pH was observed. The effect of cultivation temperature on the antifungal production was examined at different temperatures starting from 25 to 60^0C with 5^0C intervals.

H. Effect of Incubation period on antibiotic peptides production

The effects of incubation period were evaluated by 24 h interval by checking the antifungal activity was also done. Antibiotic peptides, was determined as previous above. Culture optimization was carried out based on stepwise modification of the governing parameters for antibiotic peptides production and bioassay test at incubation time in hours (24, 48, 72, 96 and 120) ; pH (6.0, 7.0, 7.5, 8.0 and 9.0); carbon sources and concentrations (glucose, Cellulose, fructose, starch and sucrose) and nitrogen source (KNO_3, $NaNO_3$, $(NH_4)_2.SO_4$, NH_4NO_3, NH_4Cl, urea, casein, yeast extract and peptone) in three replicates. Fermentation studies were carried out in three stages. To prepare inocula (b), a loopful spore from a well-sporulated plate added each 40.0 ml respective seed medium in 250 ml Erlenmeyer flasks and incubated at 28.0°C for 48 h on a rotary shaker (150 rpm). After optimization of the fermentation parameters, 2.0 ml of the seed culture (5.0%, v/v) was transferred to 250 ml Erlenmeyer flask containing 40.0 ml of the production medium. The yield of the antifungal metabolite was monitored in terms of arbitrary units (AU). Antifungal metabolite production was carried out in 100 ml starch casein medium (starch 1%, casein 0.1%, KH2PO4 0.05%, MgSO4.7H2O 0.05%, *p*H- 7) in 500 ml Erlenmeyer flasks.

I. Formulation

The oil formulation was prepared using 0.01% as glycerol oil, soybean oil as well as sugar as methylcellulose (CMC) and D-glucose at 0. 1% concentration. The liquid formulations were evaluated initially during storage periods at room temperature (25±1). The percent of survival bacteria were measured during three months.

J. Field experiments

Field experiment was conducted at El-Kanater El-Khaireia , Qalubeia governorate Egypt. The promising liquid formulation of bacteria were applied under field conditions to study their effect gray mould disease as post harvest disease of strawberry fruits during season 2010 and 2011. Field experiments were conducted under natural infection in plots (4x8 m) each comprised of 8 rows (32 holes / row) in a randomized complete block design with three replicates (plots) for each treatment. *B. brevis* and

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

B. polymyxa were cultured into broth medium and incubated on an incubator shaker (150 rpm) at 28 °C for 3. The liquid suspension of each bioagent was collected and adjusted at the concentration of 5 X 10^5 colony forming unit. Fungicide (Redomil – plus at 2 g / l) and water were used as control treatments . All treatments were applied as foliar application on strawberry plants after 50 and 70 days of transplanting meanwhile, fungicide every 15 days up to 120 days of planting. Treatments were arranged in completely randomized block, design with 5 replicates per treatment and 500 plants per replicate.

K. Preharvest evaluations for Botrytis Fruit Rot

In 2010 and 2011, all ripe and diseased fruit were harvested and graded twice weekly from 1 December to May. All fruit were harvested twice a week, but only graded once a week. The percentage of infection and severity were measured continuously during growth period.

L. Post harvest evaluations for Botrytis Fruit Rot

Three days after second application with bioagents strawberry fruits from each treatment were harvest and transferred to plant pathology Department, National Research Centre. Each treatment were divided into two groups, first stored under natural infection and second stored under artificial infection to study their efficacy against gray mould disease of strawberry fruits (12).

M. Effect of bioagents on gray mould diseases of strawberry fruits under natural infection

Strawberry fruits from each treatments were stored under natural infection (18± 1˚C) for 7 days. Treated fruits were stored inside carton trays (40x25x10cm) .All trays were stored at 18± 1˚C for 7 days**.** Gray mould disease was recorded after 7 days of storage

N. Effect of bioagents on gray mould diseases of strawberry fruits under artificial infection

Preparation of spore suspension: Spore suspensions of *B. cinerea* were prepared by inoculated sterilized PDA medium with disk (6 mm diameter) taken from 10 days old cultures of *B. cinerea* . Plates were **detabucni** at 18± 1˚C and spores suspension (10^6 spores / ml) of *B. cinerea* were prepared .

Inoculation of strawberry fruits: Fresh strawberry fruits apparently free from physical damage and diseases were used. Fruit inoculation was carried out by spraying fruits with spore suspension (10^6 spores/ml) of *B. cinerea* then air dried at room temperature 23-25C°. Treated – inoculated fruits were stored inside carton trays) 40x25x10cm) .All trays were stored at 18± 1˚C for 7 days.

Disease assessment : The percentage of disease incidence and rotted tissue part was recorded after 7 days of storage.

Statistical analysis:

Tukey test for multiple comparison among means was utilized (11).

III. RESULTS

***In vitro*, antifungal activity of *Brevibacillus brevis* and *Bacillus polymyxa* against *B. cinerea* .**

On PDA medium, strong inhibition or clearance zones were observed in the assays conducted (Fig. 1). *Brevibacillus brevis* exhibited the maximum pathogen suppression (44.4 mm) forward by *Bacillus polymyxa* by 37.7 mm. The antifungal activity of filtrate at 30 and 50 % concentrations on spore germination and growth reduction of *B. cinerea* is shown in Fig. 1. Filtrate at 30 % concentrations markedly inhibited mycelial growth of *B. cinerea*. Filtrate completely inhibited spore germination and growth of *B. cinerea* at 50% .

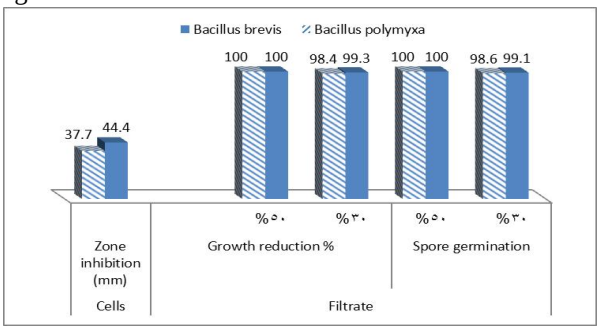

Fig. (1). In vitro, antifungal activity of Brevibacillus brevis and Bacillus polymyxa against B. cinerea

Separation and Purification of Various Fractions

The yield of antibiotics from *BCAs* was studied (data not showed). Gramicidin S and polymyxin B antibiotics were purified from culture filtrates of *B. brevis* and *P. polymyxa*, respectively using HPLC. A total of 75 and 80 ug /L of gramicidin S and polymyxin B respectively was yielded.

Optimization of incubation period, pH and carbon and nitrogen sources concentration for antibiotic peptides production

brevis: Results in figure 2 indicated that the maximum optical density obtained was at 36 hrs, and after 48 hrs incubation the curve declines. This is confirmed in the pH profile, where the pH decreases till 5.5 at 36 hrs then starts to increases again after 48 hrs incubation. The time course for dry weight in agreement with the previous observation shows that the maximum dry weight reached at 36 hrs then after 48 hrs the value of dry weight decreases with time and almost stabilizes after 66 hrs incubation time. The residual substrate profile explains the dry weight pattern, as the substrate concentration decreases gradually with time and then after 48 hrs there is negligible change in the substrate concentration. The inhibition zone curve, *Botrytis cinerea* follow the same pattern whereas the inhibition zone increase with incubation period till 48 hrs then starts to decrease (Fig.2). Production of antibiotic germicidin S in agreement with the previous observation shows that the maximum inhibition zone is reached maximum after 48 hrs, then starts to decreases with time. From all these, we deduce that: the maximum incubation period for the growth of *B. brevis* is 48 hrs. the production is growth associated (Fig.2).

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

From the experimental results on the growth, inhibition activity and production of antibiotic of *Bacillus brevis* it was noticed that by using 5 different types of carbon source, the biomass growth was minimum with glucose as substrate while the zone inhibition (cm) for *Botrytis cinerea* was maximum after 24 h incubation (Fig. 3). Also, the production of Antibiotic Gramicidin S was minimum with the cellulose as a carbon source and maximum with the fructose as a carbon source (Fig. 3). So, the cellulose was chosen as an optimum carbon source for *B. brevis.* By using different concentration of cellulose for the growth of *B. brevis*, pH was minimum at 10 and 30 g/l glucose concentration and maximum at 15, 20 and 25 g/l at the end of fermentation time (Fig. 4). The dry weight concentration increased with increasing the glucose concentration. Also, the production of Antibiotic Gramicidin S increased by increasing the cellulose concentration till 20 g/l then decreased again. The zone inhibition for *Botrytis cinerea* increased gradually by increasing the cellulose concentration. From the previous observations, 20 g/l cellulose concentration was chosen as the optimum glucose concentration (Fig. 4). By using different types of nitrogen source for the growth of *B. brevis* it was noticed that the maximum biomass growth was achieved with soyabean, while the maximum production of Antibiotic Gramicidin S and the zone inhibition of *Botrytis cinerea* were obtained with Trypton as a nitrogen source (Fig. 5). So, the optimum nitrogen source chosen was trypton.

Polymxya

Results in figure (6) indicated that the maximum optical density was obtained at 36 hrs and then remains constant. This also is confirmed in the pH profile, where the pH decreases till 5 at 36 hrs then remains constant for the rest of incubation time. The time course for dry weight varies slightly from the previous observations, whereas the maximum dry weight is reached at 48 hrs then almost stabilizes for the remaining time. The residual substrate In addition, D-glucose at 1% as sugar sources also gave better effect. However, the other formulation gave high effect in compared with untreated control .So; both glycerol oil plus D-glucose were used in the fermination

Formulation

Analysis of each formulation revealed that glycerol oil at o.o1% the best oil. Used for protect bacteria for 3 months in compared with untreated control (Fig. 10).

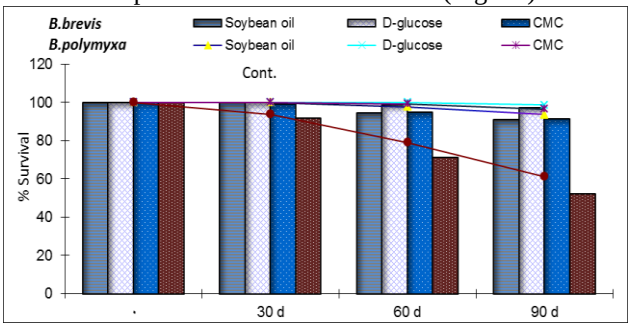

Fig. 10. Effect of liquid formulation on the survival of bacteria during storage periods

and formulation process. Concentration. In the inhibition zone curve, *Botrytis cinerea* follow the same pattern whereas the inhibition zone increases with incubation period till 48 hrs then starts to decrease. profile explains the dry weight pattern, as the substrate concentration decreases gradually with time and then after 48 hrs there is negligible change in the substrate Production of antibiotic polymxin B increased radically from the beginning of incubation time till 48 hrs then almost stabilize. From all these, we deduce that, the maximum incubation period for the growth of *B. polymxya* is 48 hrs. The production is growth associated (Fig. 6). From the experimental of different carbon sources on the growth, inhibition activity and production of antibiotic of *P. polymyxa* results it was noticed that by using 5 different types of carbon source, the biomass growth was minimum with glucose as substrate while the zone inhibition (cm) for *Botrytis cinerea* were maximum (Fig. 7). Also, the production of Antibiotic Polymyxin B was maximum with the glucose as a carbon source (Fig. 7). By using different concentration of glucose for the growth of *P. polymyxa*, pH was minimum at 25 and 30 g/l glucose concentration and maximum at 10, 15 g/l at the end of fermentation time. The dry weight concentration decreased with increasing the glucose concentration till 20 g/l then started to increase. Also, the production of Antibiotic Polymyxin B increased by increasing the glucose concentration till 20 g/l then decreased again (Fig. 8). The zone inhibition for *Botrytis cinerea* also increased gradually by increasing the glucose concentration till 20 g/l then decreased. From the previous observations, 20 g/l glucose concentration was chosen as the optimum glucose concentration. So, the glucose was chosen as an optimum carbon source for *P. polymyxa*..By using different types of nitrogen source for the growth of *P. ploymyxa* it was noticed that the maximum biomass growth was achieved with malt extract, while the maximum production of Antibiotic Polymyxin B was obtained with malt extract and trypton (Fig. 9). The maximum zone inhibition of *Botrytis cinerea* were obtained with malt extract as a nitrogen source. So, the optimum

Field tests

The results of field application of D-glucose | glycerol oil formulation of bacteria indicated that both bacteria were as effective as the chemical fungicide Redomil – plus at concentration of 2 g / l for the control of strawberry gray mould. On the preharvest, there was very effective protection against strawberry gray mould using *Bacillus brevis*, which was statistically different and better than the chemical fungicide Redomil – plus and the absolut control (Fig.11). However, *Bacillus polymyxa* could also effectively control grey mould caused by *B.cinerea*. It is evident from the data in Fig. that applying strawberry with either *Bacillus brevis* or *Bacillus polymyxa* significantly improved fruit quality in terms of increasing fruit weight, total soluble solids, and yield compared with fungicide and untreated control. Results further indicate

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

that both bacteria had no significant differences in improving fruit quality of tested cultivar. So, the two bacteria studied compounds had relatively similar effects in this connection.

Biocontrol efficacy of Bacillus brevis and Bacillus polymyxa in non-inoculated strawberry fruits

Our experiments evaluated the efficacy of bacteria antagonist in reducing the natural development of decay following storage at 18 ^{0}C for 7 days. Biocontrol efficacy of the antagonistic *Bacillus brevis* and *Bacillus polymyxa* to control *B. cinerea* in non-inoculated strawberry fruits was showed in Fig . 10. Both antagonistic bacteria treatment reduced the disease incidence of grey mold rot in strawberry fruits (Fig.11) . After 7 d of incubation at 18± 1˚C , fruits treated with *Bacillus brevis* showed better control for *B.cinerea* than those treated with fungicide . *Bacillus polymyxa* could also effectively control decays caused by *B.cinerea* at 18± 1˚C.

Biocontrol efficacy of Bacillus brevis and Bacillus polymyxa against B. cinerea in inoculated strawberry fruits

Our experiments evaluated the efficacy of bacteria antagonist in reducing the artificial development of decay following storage at 18 ^{0}C for 7 days .Biocontrol efficacy of the antagonistic *Bacillus brevis* and *Bacillus polymyxa* to control *B. cinerea* in inoculated strawberry fruits was showed in Fig . 12. After 7 days of incubation at 18± 1˚C , fruits treated with *Bacillus brevis* showed better control for *B. cinerea* . *Bacillus polymyxa* also effectively control decays caused by *B. cinerea* at 18± 1˚C in compared with fungicide and control treatment.

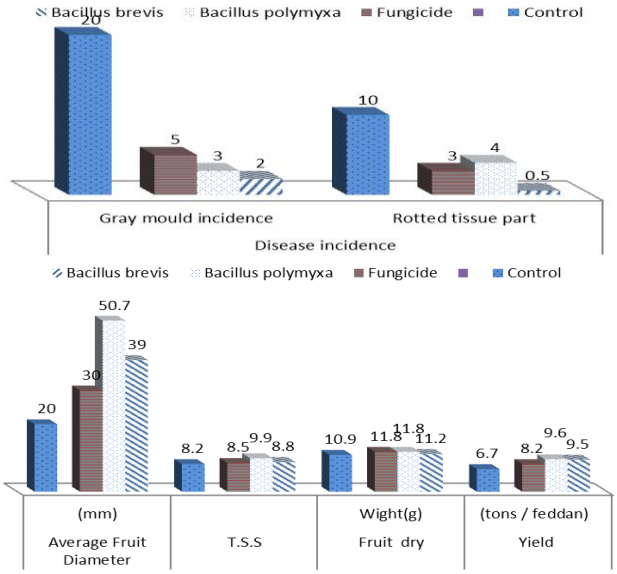

Fig. 11. Effect of preharvest treatments with *Brevibacillus brevis* and *Bacillus polymyxa* on gray mould incidence and rotted tissue part of strawberry fruits under natural infection and fruit yield in vivo.

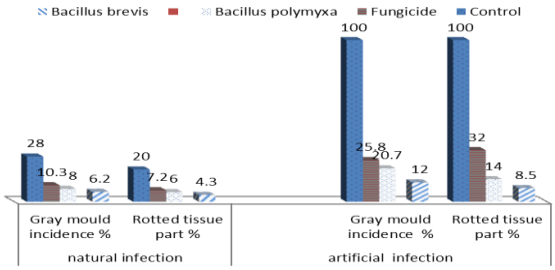

Fig. 12 Effect of preharvest treatments with *Brevibacillus brevis* and *Bacillus polymyxa* on gray mould incidence and rotted tissue part of strawberry fruits under natural and artificial infection of post harvest.

IV. DISCUSSION

Gray mold, caused by Botrytis cinerea is one of the important strawberry diseases that causes losses before or after harvest wherever strawberry (2; 17**).** Although B . cinerea can infect almost all aerial plant parts, the most damaging infections occur at flowering stage (10). Numerous studies have described the isolation of micro-organisms and demonstrated their potential to antagonize. Many studies have investigated the potential of BCAs as B. cinerea antagonists in a wide range of fruit crops, including tomatoes (9), strawberries (3) and pears (12). BCAs with reported activity against B. cinerea on grape tissues include Bacillus spp. (14), Brevibacillus brevis (formerly Bacillus brevis; Seddon et al., 15). Other species of Bacillus , Paenibacillus polymyxa (formerly Bacillus polymxa) a common bacterium belongs to the group of plant growth promoting rhizobacteria (PGPR) cable of suppressing of plant diseases (7). In this study we investigated whether it is possible to use phyllosphere Bacillus brevis and Bacillus polymyxa biological control agents which produce a high yield of peptides antibiotics, can also be used for the development of new biological control agents against Botrytis grey mould disease on strawberry as pre and post harvest. The results of the present experiments showed that, both tested BCAs effective to reduce the linear growth and inhibited spore germination of Botrytis. The mode of action of Bacillus brevis and Bacillus polymyxa against B. cinerea infection of fruits has been suggested to be secretion of antibiotics peptides. B. brevis has been shown to produce a single cyclic as decapeptide antibiotic gramicidins S which has been shown to be fungicidal (7). Bacillus polymyxa is known to produce peptide antibiotics polymyxin B (7). Polymyxin B, functions as a cell membrane inhibitor and disorganizes the structure or inhibits the function of bacterial membranes. Based on the efficacy to reduce gray mould in a preliminary field test and during storage, a submerged fermentation production process for Bacillus brevis and Bacillus polymyxa was further developed and scaled-up production. However, this procedure is impractical under commercial conditions and therefore the microorganisms should be formulated in order to be able to store and transport them without drastic losses in viability. One way to decrease the amount of micro-organisms

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

needed for practical application in the field is to combine the biological approach with the application of glycerol, not only in preharvest but also in post harvest. Thus our results should encourage the use of *Bacillus brevis* and *Bacillus polymyxa* on a large scale for biocontrol of pre and post harvest strawberry. Moreover, our results showed that biological control is as efficient as the best fungicide available.

ACKNOWLEDGMENT

This project was supported financially by the Science and Technology Development Fund (STDF), Egypt, Grant No 216 under title: Development of Bioproducts as Bio-fungicides for Controlling of Major Foliar Diseases of Some Economic Horticultural Crops, from 2009- 2012 ; PI. Wafaa M. Haggag

REFERENCES

[1] P. A. H. M. Bakker, Pieterse, C. M. J., and van Loon, L. C.(2007). Induced systemic resistance by fluorescent Pseudomonas spp. Phytopathology 97:239-243.

[2] Y . Elad, Williamson B, Tudzynski P, Delen N (2004). Botrytis spp. And diseases they cause in agric. Systems-an introduction. In: Elad Y, Williamson B, Tudzynski P, Delen N (eds). Botrytis: Biology, Pathology and Control. Kluwer Academic Publishers, Netherlands, pp. 1-8.

[3] R,Guetsky, Shtienberg D, Elad Y, Fischer E, Dinoor A.(2002). Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. Phytopathology 92, 976–85.

[4] J. Kovach, Petzoldt, R. and Harman, G.E. (2000). Use of honey bees and bumble bees to disseminate Trichoderma harzianum 1295-22 to strawberries for botrytis control, Biol. Control **18**.

[5] M. Haggag, Wafaa (2002). Sustainable Agriculture Management of Plant Diseases. Online Journal of Biological Science. 2: 280-284.

[6] M. Haggag, Wafaa (2003). Biological control of plant diseases, and its developments. Prospects of the Recent Agricultural Research. The Second Scientific Conference, National Research Center, Egypt. Pp: 45.

[7] M. Haggag, Wafaa (2008). Isolation of bioactive antibiotic peptides from Bacillus brevis and Bacillus polymyxa against Botrytis grey mould in strawberry. Archives Journal of Phytopathology and Plant Protection (German) . 41(7): 477 – 491.

[8] Z. Ma and Michailides, T. 2005. Genetic structure of Botrytis cinerea populations from different host plants in California. Plant Dis. 89: 1083-1089.

[9] R. McHugh , White D, Schmitt A, Ernst A, Seddon B, 2002. Biocontrol of Botrytis cinerea infection of tomato in unheated polytunnels in the North East of Scotland. Bulletin OILB/ SROP 25, 155–8.

[10] C.K. Myresiotis, Karaoglanidis GS, Tzavella-Klonari K (2007). Resistance of Botrytis cinerea isolates from vegetable crops to anilinopyrimidine, phenylpyrrole, hydroxyanilide, benzimidazole, and dicarboximide fungicides. Plant Dis. 91: 407-413.

[11] J. Neler, Wassermann, W. and Kutner, M.H. 1985. Applied linear statistical models. In: Richard, D. (ed) Regression Analysis of Variance and Experimental Design: 2nd Irwin Inc. Homewood Illionois. pp: 117-155.

[12] C. Nunes, Usall J, Teixido N, Vinas I, 2001. Biological control of postharvest pear diseases using a bacterium, Pantoea agglomerans CPA-2. International Journal of Food Microbiology 70, 53–61.

[13] S. Pande , Sharma, M.; , KishoreK.; Shivram, L. and Mangala, N. 2010. Characterization of Botrytis cinerea isolates from chickpea: DNA polymorphisms, cultural, morphological and virulence characteristics. African Journal of Biotechnology, 97961-7967.

[14] B. Paul, Chereyathmanjiyil A, Masih I, Chapuis L, Benoit A, 1998. Biological control of Botrytis cinerea causing grey mould disease of grapevine and elicitation of stilbene phytoalexin (resveratrol) by a soil bacterium. FEMS Microbiology Letters 165, 65–70.

[15] B. Seddon, McHugh RC, Schmitt A, 2000. Brevibacillus brevis – a novel candidate biocontrol agent with broadspectrum antifungal activity. In: Proceedings of the Brighton Conference.

[16] B. Willamson , Tudzynski, B. and Vankan, L. 2007. Botrytis cinerea: the cause of grey mould disease. Molecular Plant Pathology, 8: 561–580.

[17] H. Zhang, Wang, L., Dong, Y., Jiang, S., Cao, J., Meng, R., 2007. Postharvest biological control of gray mold decay of strawberry with Rhodotorula glutinis. Biological Control 40 (2), 287–292.

AUTHOR BIOGRAPHY

First Author Department of Plant Pathology, National Research Centre –Egypt, wafaa_haggag@yahoo.com;

Second Author: Department of Plant Pathology, National Research Centre –Egypt.

Third Author: Department of Vegetable Research, National Research Centre –Egypt.

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

APPENDIX

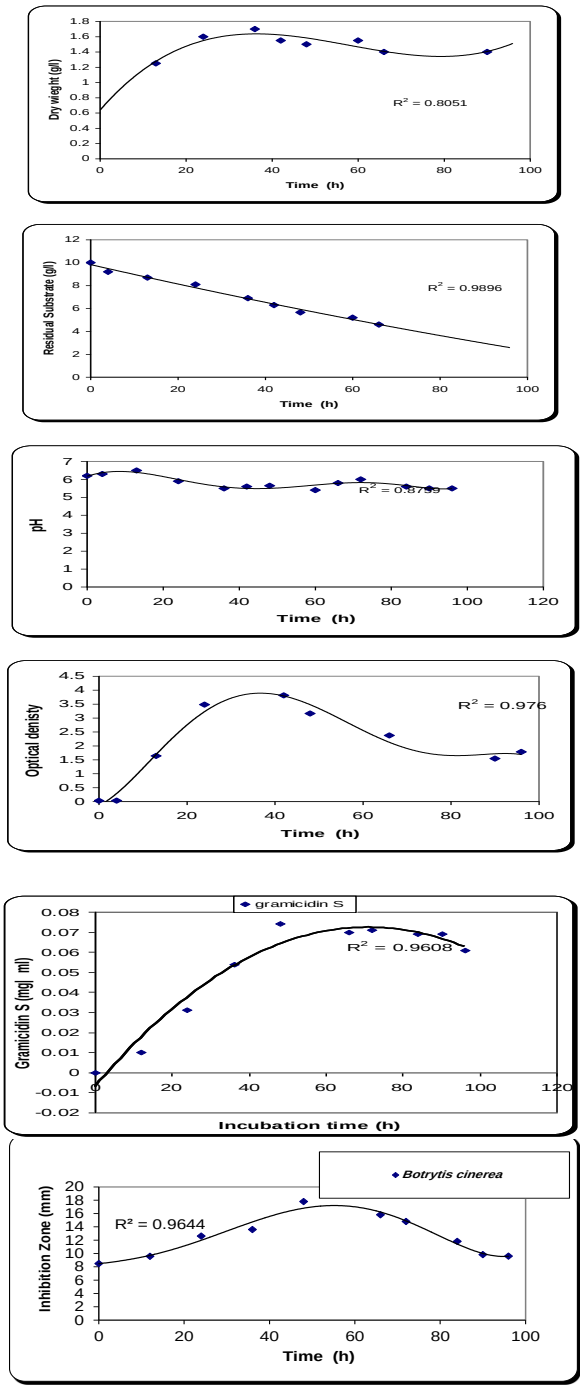

Fig . 2. Optimization of incubation time for reduction of pathogens growth and production of antibiotic by *B. brevis*.

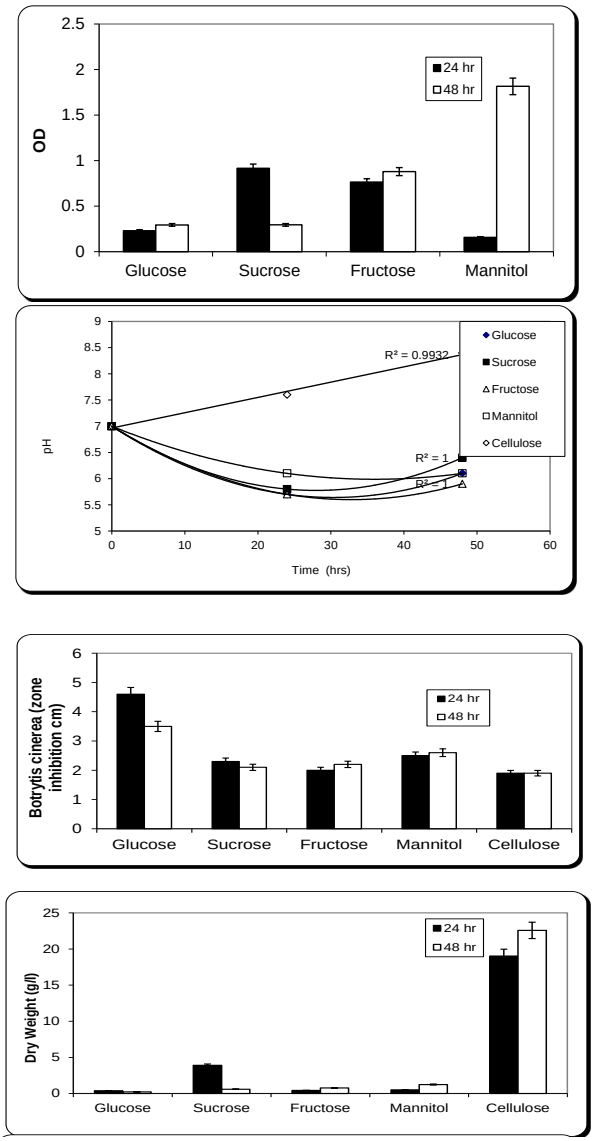

Fig. 3 : Effect of different carbon sources on the growth, inhibition activity and production of antibiotic. of *Bacillus brevis*

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

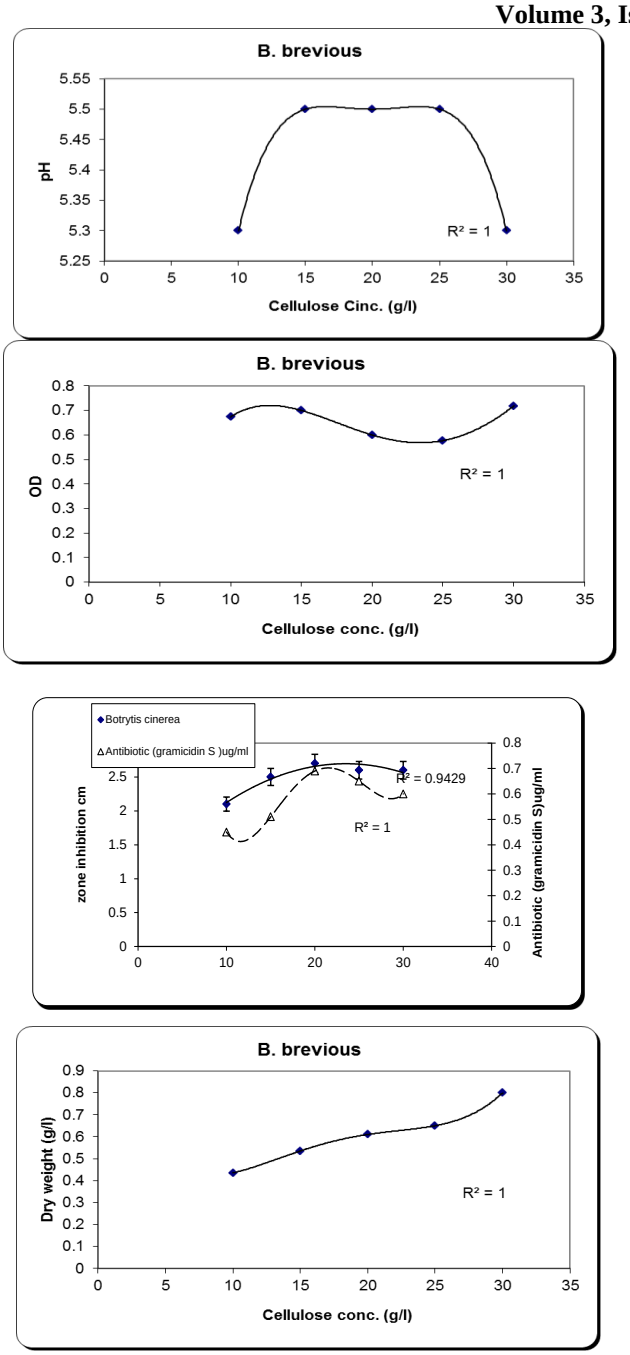

Fig. 4: Effect of different concentration of cellulose on the growth, inhibition activity and production of antibiotic of *Bacillus brevis* .

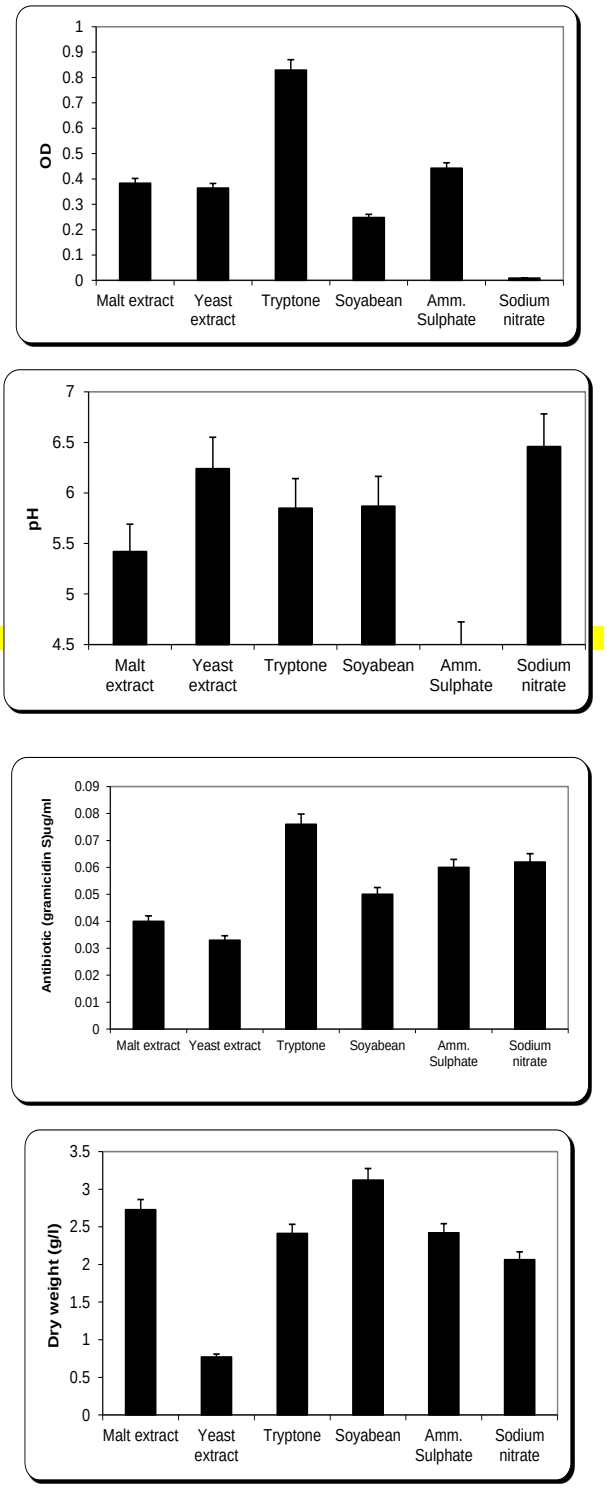

Fig. 5 : Effect of different Nitrogen sources on the growth, inhibition activity and production of antibiotic of *Bacillus brevis*

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

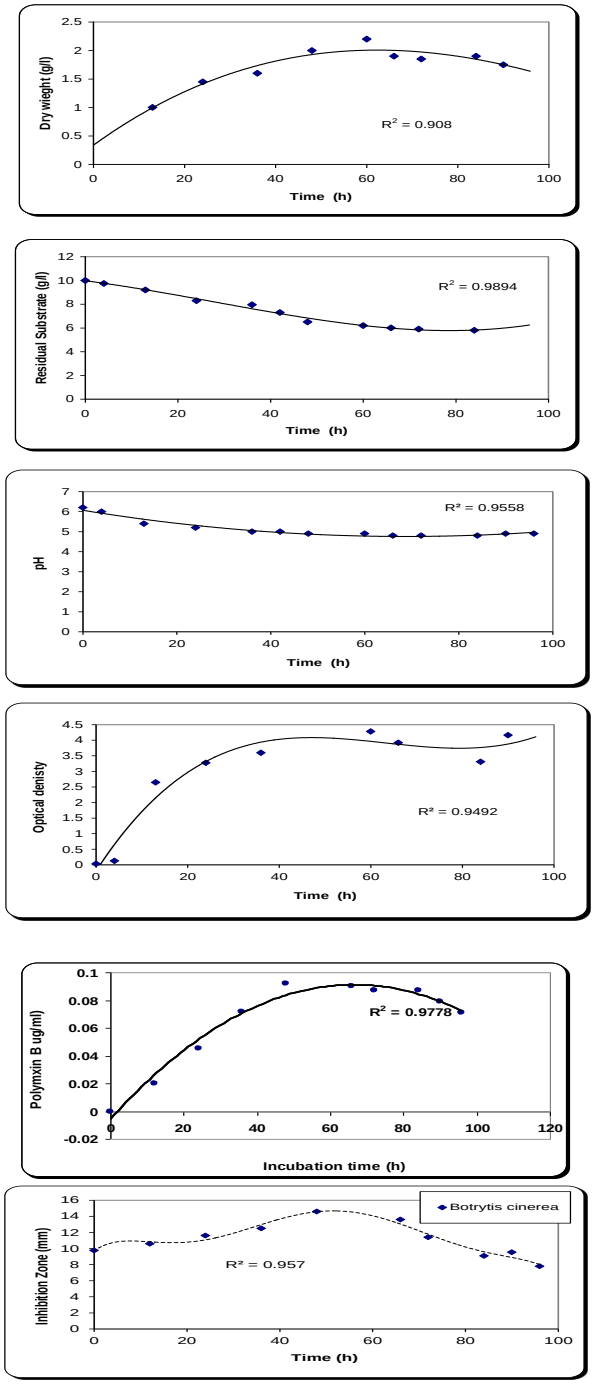

Fig . 6. Optimization of incubation time for reduction of pathogens growth and production of antibiotic by *P. polymyxa*.

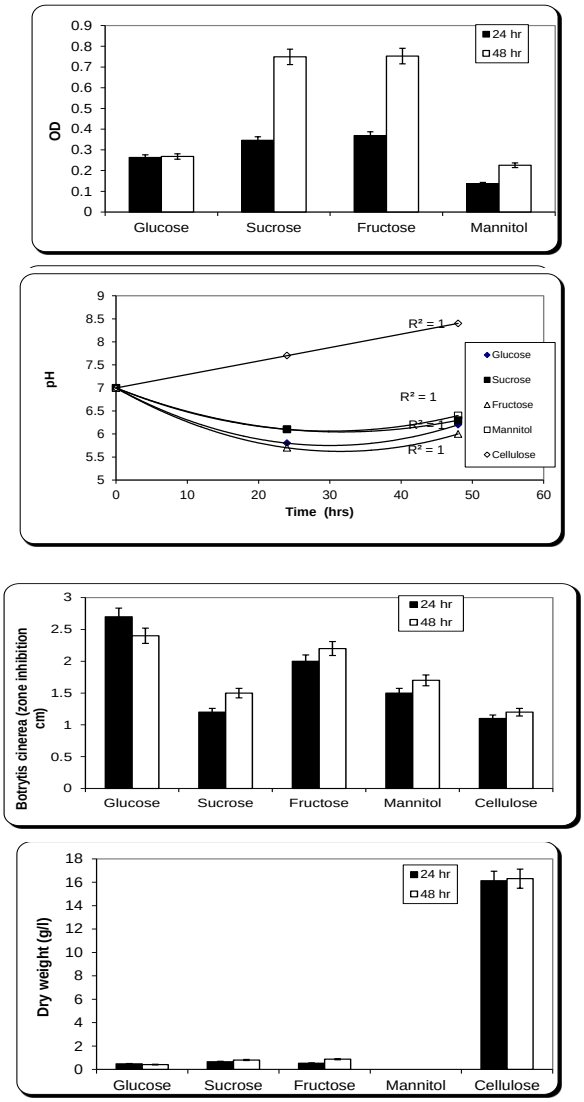

Fig. 7.: Effect of different carbon sources on the growth, inhibition activity and production of antibiotic. of *Paenibacillus polymyxa*

ISSN: 2277-3754
ISO 9001:2008 Certified
International Journal of Engineering and Innovative Technology (IJEIT)
Volume 3, Issue 2, August 2013

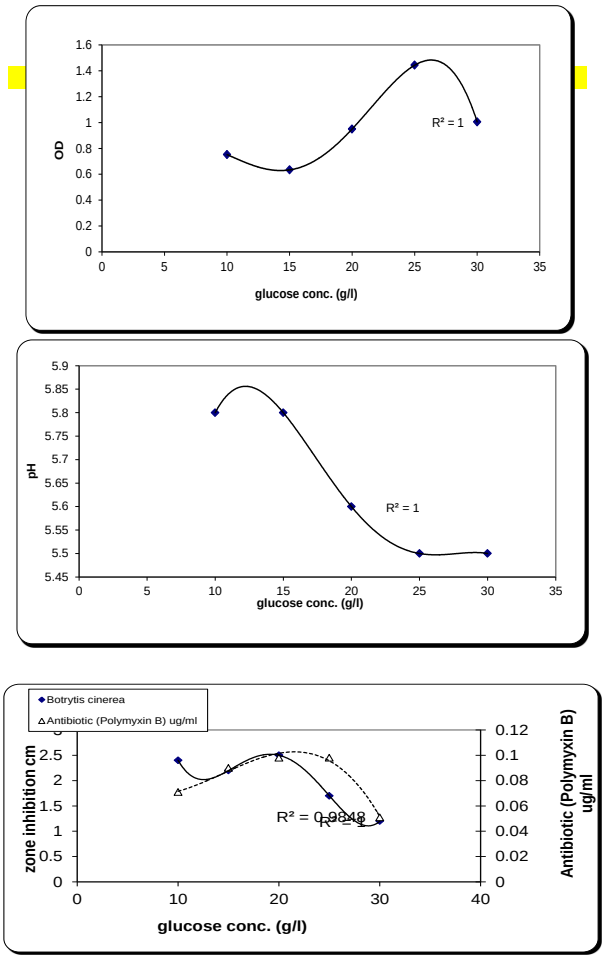

Fig. 8. Effect of different carbon source concentrations on the growth, inhibition activity and production of antibiotic of *P. polymyxa*

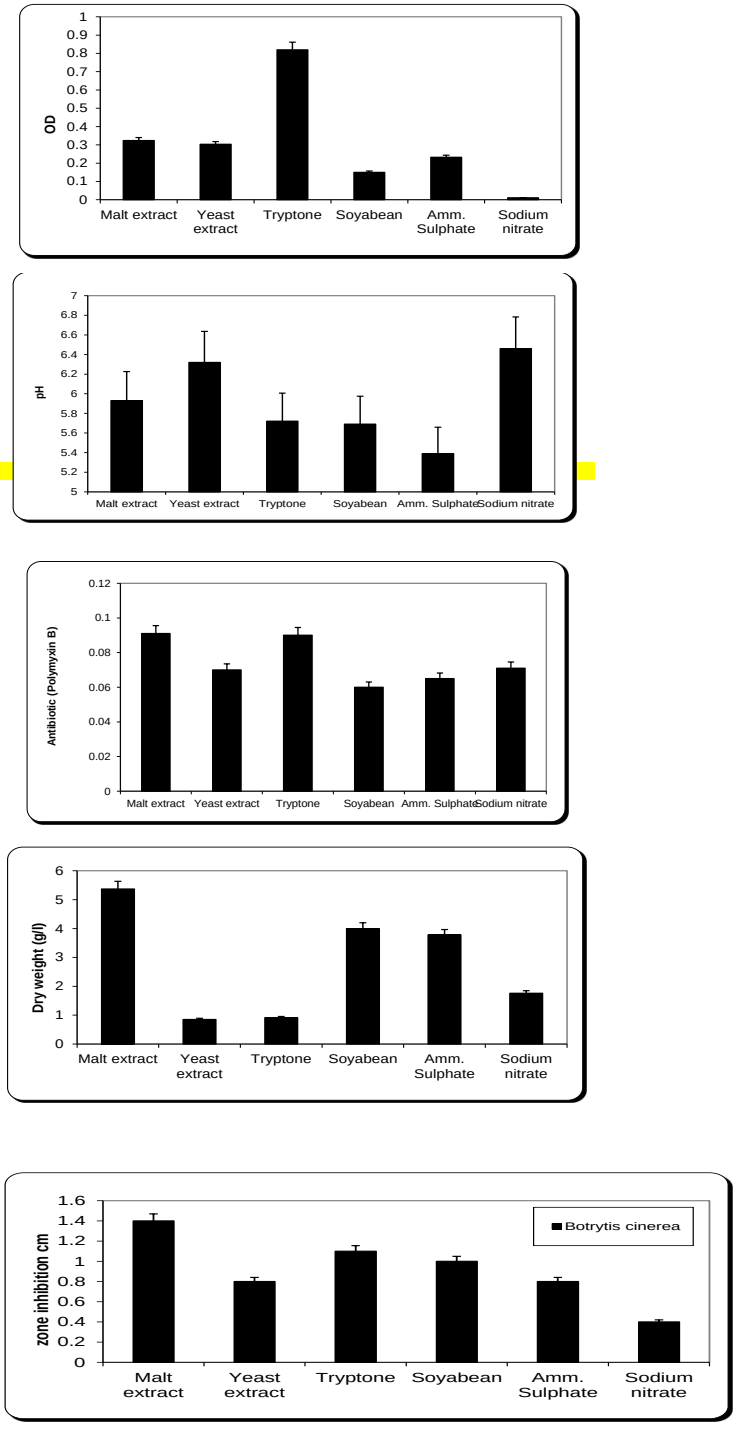

Fig. 9. Effect of different nitrogen source on the growth, inhibition activity and production of antibiotic of *P. polymyxa*