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Effect of salt-tolerant PGPR on the activity of some microbial and plant enzymes under saline stress

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ABSTRACT

This experiment was conducted under greenhouse conditions using a randomized complete block design (RCBD) with three replicates in the experimental farm station of Fac. Agric. at Moshtohor during 2011 season.This research aims to study the effect of tomato and pepper inoculation with salt-tolerant PGPR strains (*Pseudomonas fluorescence D23*, *Bacillus pumilus D135* and *Azospirillum lipoferum D178*) for tomato while, (*Bacillus megaterium* D159, *Paenibacillus alvie* D139 *and Azospirillum lipoferum* D207) for pepper in combination with humic acid and organic manure (compost) on some microbial enzymes activity such as dehydrogenase, phosphatase, nitrogenase and oxidative enzymes like nitrate reductase, peroxidase and polyphenol oxidase. The application of salt-tolerant PGPR in combination with compost at a rate of 10 ton/fed. and humic acid at a rate of 4 kg/fed. gave positive impact on the tested enzymes which resulted to beneficial effect on tomato and pepper cultivated under saline stress.

Key words: PGPR, saline stress, oxidative enzymes, microbial enzymes, pepper and tomato.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are one group of microorganisms which are beneficial to crops. PGPR are a heterogeneous group of soil microorganisms and they can be found in the rhizosphere, most of PGPR are free living or associative (Tilak *et al.*, 2005). PGPR have positively influence plants vitality and the ability of the plants to cope with a biotic stress conditions such as drought and salinity (Woitke *et al.*, 2004).

Under salt stress, PGPR have shown positive effects in plants such germination rate, tolerance to drought, weight of shoots and roots, plant growth and yield (Kokalis-Burelle *et al.*, 2006).

Tomato is a major vegetable crop that has achieved tremendous popularity over the last century, it is practically grown in every country of the world - in outdoor fields, greenhouses and net-houses. Also, pepper is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent source of natural colors and antioxidant compounds (Navarro *et al.*, 2006). Mittova *et al.* (2002) reported that there was correlation between salt tolerance and increase the activity of anti-oxidant system in vegetable crops.

MATERIALS AND METHODS

Soil and soil mixtures

Soil of the experiment was obtained from El-Sharkia Governorate (Sahl El-Hussinia). The obtained soil was mixed with Agric. gypsum at rate of ½ ton/fed. and course sand at rate of 40 ton/fed., then subjected to mechanical and chemical analyses before using in cultivation (Table 1). The analysis was achieved in Analysis Center and Agricultural Consultancy, Fac. Agric. at Moshtohor according to the method described by Page *et al.* (1982).

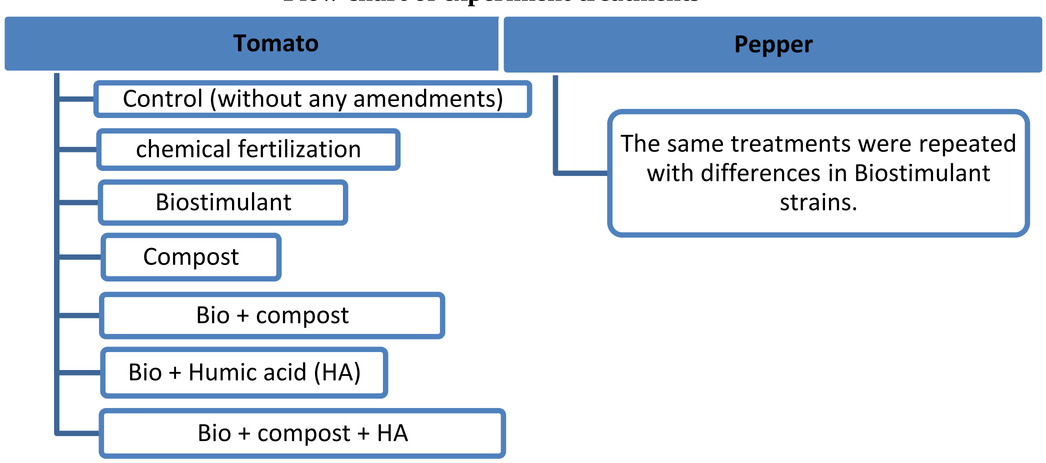
Table (1).Mechanical and chemical analyses of the experimental soil.

Mechanical analysis				Chemical analysis											
								Soluble cations (meq./L)				Soluble anions (meq./L)			
Sand (%)	Silt (%)	Clay (%)	Textural class	EC (ds/m)	pH	Organic matter (%)	Total nitrogen (%)	Na^+	K^+	Ca_2^+	Mg^{2+}	$CO_3^=$	HCO_3^-	Cl^-	SO_4^{2-}
Soil before adjustment															
45.01	16.32	38.67	Clay	24.34	8.30	0.62	0.010	201	1.34	14.22	27.12	zero	9.93	186	47.75
Soil after adjustment															
50	33	17	Clayey silty	13.92	8.28	1.62	0.114	57.93	8.27	35.0	38.0	zero	28.0	81.0	30.2

Experimental design

Treatments were distributed in a randomized complete block design with three replicates.

Flow chart of experiment treatments



Preparation of biostimulant inocula

The biostimulant inocula for tomato (*Pseudomonas fluorescence D23*, *Bacillus pumilus D1395,and Azospirillum lipoferum D178*) *and* pepper biostimulant (*Bacillus megaterium D159*, *Paenibacillus alvie D135*and *Azospirillum lipoferum D207*) were prepared in specific broth media.

Cell suspension of *A. lipoferum* contains about (10 x 10^5cfu/ml) 7 days-old on semi-solid malate medium (Dobereiner, 1978), *Bacillus megaterium* (90 x 10^6 cfu/ml) 2 days-old on modified Bunt and Rovira agar medium, modified by (Abdel-Hafez, 1966) , *Bacillus pumilus* (90 x 10^6 cfu/ml) 2 days-old, *P. alvie* (60 x 10^7cfu/ml) 2 days-old and *Ps. fluorescence* (20 x 10^6cfu/ml) 5 days-old on King's medium (King *et al.*, 1954).

Cultivation process

Prior to cultivation, tomato and pepper seedlings were soaked by dipping the root system in a mixture of PGPR inocula (cell suspension of biostimulant) for 60 minutes before transplanting; sucrose solution (40 %) was used as an adhesive agent. The same prepared PGPR inocula were added to grown plants three times throughout the growing season at a rate of 300 ml/pot. The recommended dose of compost was 10 ton/fed for vegetable crops. Whereas, humic acid was added to soil at rate of 4 kg/fed. compost and humic acid were added at transplanting. A half dose of inorganic nitrogen fertilizer (50 kg N/fed) as ammonium sulphate was supplemented for treatments of biostimulant and biostimulant + humic acid. Also, a full dose of inorganic phosphorus fertilizer (25 kg P_2O_3/fed) as calcium super-phosphate and potassium fertilizer (40 kg K_2O/fed) as potassium sulphate were supplemented for all treatments in three equal doses.

Determinations

Microbial enzymes

Dehydrogenase activity was assayed in soil according to Glathe' and Thalmann (1970), phosphatase activity was estimated according to Drobnikova (1961) and Nitrogenase activity was measured by using the acetylene reduction technique given by Diloworth (1970).

Oxidative enzymes

Nitrate reductase was determined using the method of Abdel-Samad *et al.* (2004),peroxidase activity was determined according to the method described by Allam and Hollis (1972) andpolyphenol oxidase activity was determined according to the method described by Matta and Dimond (1963).

Statistical analysis

Statistical analysis was carried out according to Snedecor and Cochran (1989). The differences between the means value of various treatments were compared by Duncan's multiple range test (Duncan's, 1955).

RESULTS AND DISCUSSION

Microbial enzymes activities

Dehydrogenase activity

Data presented in Table (2) indicated that rhizosphere of tomato cultivated in moderately saline soil with no amendments (control) gave lowest DHA values followed by soil amended with chemical fertilizer, this result may be due to the high salts concentration which decreased the microbial activities. DHA in various treatments were significantly higher at flowering stage (60 days) than vegetative one. This increase of DHA could be attributed to the beneficial effect of root exudates which increase during flowering stage, similar trend of results was observed with pepper treatments. Data also revealed that the inoculation of tomato with biostimulant combined with humic acid and compost being 84.5 µg TPF/ g dry soil therefore it increased with **1.2 fold comparing to biostimulant** treatment. This trend of results was observed also with pepper. This is likely be due not only to the promotion effect of biostimulant on microbial proliferation but also to the beneficial effect of compost and humic acid. Liu *et al.* (1992) reported that the addition of humic acid to soil enhanced DHA at vegetative and flowering stages. This result could be attributed to the synergistic effect between biostimulant and natural microbial flora occurred in compost which increased the microbial respiration rate.

Table 2. Periodical changes in dehydrogenase activity in moderately saline soil cultivated with tomato and pepper.

Treatments	DHA activity as µg TPF/ g dry soil				
		Periods (day)			
	Initial	15	30	60	120
			Tomato		
Control	8.1^{e}	13.2^{g}	29.5^{e}	39.9^{f}	37.3^{e}
Chemical fertilization	9.0^{d}	15.9^{f}	28.3^{e}	57.1^{e}	52.3^{d}
Biostimulant	13.6^{bc}	17.4^{e}	48.8^{c}	68.2^{c}	58.8^{c}
Compost	15.5^{b}	20.7^{cd}	46.2^{d}	84.5^{b}	75.2^{a}
Bio. + compost	18.3^{ab}	27.8^{a}	56.8^{b}	86.5^{a}	72.8^{b}
Bio. + HA	13.1^{c}	19.2^{d}	25.5^{f}	62.5^{d}	52.5^{d}
Bio. + compost + HA	18.7^{a}	24.5^{b}	64.5^{a}	86.8^{a}	74.5^{a}
			Pepper		
Control	10.1^{e}	22.2^{d}	33.5^{d}	59.9^{d}	41.3^{e}
Chemical fertilization	15.0^{c}	21.1^{d}	34.1^{cd}	58.4^{d}	42.9^{e}
Biostimulant	17.4^{b}	22.2^{d}	36.1^{c}	77.6^{c}	48.2^{d}
Compost	10.4^{e}	27.1^{c}	36.2^{c}	94.3^{b}	67.8^{c}
Bio. + compost	12.9^{d}	33.1^{b}	66.4^{b}	95.0^{b}	78.8^{b}
Bio. + HA	14.1^{cd}	25.4^{cd}	35.7^{cd}	52.8^{e}	42.8^{e}
Bio. + compost + HA	18.7^{a}	41.9^{a}	70.6^{a}	109.5^{a}	87.5^{a}

Control: Without any soil amendments.
HA: Humic acid

Obtained data showed that highly significant increase of DHA was observed in soil amended with compost or biostimulant compared with soil amended with chemical fertilizers. This result was observed with tomato and pepper at all the experimental periods and was in accordance with Marinara *et al.* (2000) reported that a higher DHA values was observed in soil amended with compost compared to soil fertilized with chemical fertilizers. Also, Zaghloul *et al.* (2008) who studied the efficiency of soil inoculation with *A. chroococcum* and *B. megaterium* on some soil enzymes activity and found that the combination with these PGPR strains gave higher values of dehydrogenase activity compared with individual inoculation treatments.

Treatment of pepper with biostimulant and compost was significantly increased the DHA values than soil amended with either biostimulant or compost each one solely at all determination periods except at 60 days. These results are in harmony with those obtained by Abou-Aly (2005) who found that the combined inoculation with *Azospirillum sp*. and *Bacillus sp*. increased the DHA at all growth plant stages.

The highest DHA values were observed when compost was combined with humic acid and biostimulant, this result was observed through all pepper growth stages and resulted to increase the enzyme activity at 60 days by 2.07 fold comparing to treatment with biostimulant and humic acid. This result is discrepancy with those obtained by Chen *et al.* (2004) who reported that humic acid increased the uptake of nutrients and water and stimulated the soil microorganisms.

Phosphatase activity

Data in Table (3) showed that tomato inoculation with *Pseudomonas fluorescence D23*, *Bacillus pumilus D139* and *Azospirillum lipoferum D17*8 gave significant increase of phosphatase activity compared with soil amended with compost or chemical fertilizer after 120 days. This trend of results was observed also with pepper. Similar trend of results was observed by Ponmurgan and Gopi (2006) who reported that phosphatase activity of *Pseudomonas sp.* which was isolated from rhizosphere zone had higher activity and there was a positive correlation between phosphate solubilizing bacteria and phosphatase activity.

Obtained data in Table (3) showed that the lowest values of phosphatase activity in tomato and pepper rhizosphere were observed in moderately saline soil without any amendments (control) followed by chemical fertilization treatment. This result is in agreement with Krishnakumar *et al.* (2007) who found that the application of recommended chemical fertilizer showed significant lower phosphatase activity rather than all organic manure treatments.The highest significant values of phosphatase activity were observed in soil amended with compost, HA and biostimulant strains after 15 days in tomato and at all determination periods of pepper. These results are in harmony with Takeda *et al.* (2009) who found that the application of compost in combination with phosphate solubilizing bacteria significantly increased soil microflora and soil enzymes activity such as dehydrogenase and phosphatase. Also, Bama *et al.* (2008) applied the humic acid at 20 kg/ha or 10 kg/ha with foliar spray and recorded an increase of enzymatic activities such as catalase, dehydrogenase and phosphatase. Data also showed that phosphatase activity was significantly higher at flowering stage (60 days) than vegetative one. Except the inoculation of tomato with biostimulant, phosphatase activity was gradually increased through the first 60 days of tomato growth thereafter decreased. On contrast, phosphatase activity in pepper rhizosphere reached to maximum after 120 days.

No significant differences of phosphatase activity could be detected between the control and chemical fertilization treatment before 15 days, whereas significant lower activities could be detected comparing to other treatments throughout pepper growth till 120 days.

It was clear that phosphatase activity was highest in pepper rhizosphere treated with biostimulant combined

Table 3. Periodical changes in phosphatase activity in moderately saline soil cultivated with tomato and pepper.

Treatments	Phosphatase activity as µg phosphorus/g dry soil				
		Periods (day)			
	Initial	15	30	60	120
			Tomato		
Control	12.8^{b}	17.2^{d}	26.2^{g}	50.23^{e}	46.54^{f}
Chemical fertilization	9.9^{f}	20.9^{bc}	57.4^{e}	88.30^{d}	77.16^{e}
Biostimulant	10.8^{d}	21.2^{b}	56.6^{f}	90.27^{cd}	99.40^{a}
Compost	10.1^{de}	19.2^{c}	62.4^{d}	101.7^{a}	84.53^{d}
Bio. + compost	11.5^{c}	27.8^{a}	92.1^{b}	100.1^{ab}	99.03^{a}
Bio. + HA	12.7^{b}	18.9^{cd}	81.6^{c}	91.20^{c}	88.41^{c}
Bio. + compost + HA	13.3^{a}	25.2^{ab}	97.0^{a}	97.25^{b}	96.72^{b}
			Pepper		
Control	9.8^{d}	16.6^{d}	27.5^{e}	29.5^{f}	44.57^{f}
Chemical fertilization	9.9^{d}	17.7^{c}	52.4^{d}	64.5^{e}	69.54^{e}
Biostimulant	11.8^{cd}	20.0^{bc}	65.4^{c}	71.4^{d}	99.31^{c}
Compost	12.3^{bc}	21.7^{b}	63.4^{cd}	88.5^{c}	98.22^{c}
Bio. + compost	15.3^{ab}	26.5^{a}	74.0^{b}	93.4^{b}	112.3^{b}
Bio. + HA	12.7^{bc}	17.3^{c}	63.6^{cd}	71.6^{d}	89.72^{d}
Bio. + compost + HA	17.3^{a}	26.7^{a}	92.1^{a}	98.0^{a}	114.7^{a}

Abbreviations as those stated in Table (2)

with compost and HA comparing to other soil treatments. This result may be attributed to the effect of biostimulant strains which play an important role in phosphorus availability and also to the synergistic effect between biostimulant strains and microbial community occurred in compost and also to the effect of humic acid on the proliferation of different soil microorganisms.

Nitrogenase activity (N_2-ase)

Data in Table (4) showed that nitrogenase activity was affected by the investigated treatments, tomato amended with chemical fertilizers gave the lowest values of N_2-ase activity compared to other treatments at 15, 30 and 60 days. While, no significant differences were observed N_2-ase at initial and 15 days between control and chemical fertilization treatments in pepper rhizosphere. This result is in harmony with that obtained by Anne-Sophie *et al.* (2002) who found that the addition of chemical fertilizers such as ammonium nitrate decreased the nitrogenase activity.

Higher records of N_2-ase activity were observed in soil treated with compost than biostimulant each one singularly at initial, 15 and 60 days. Whereas after 120 days of tomato growth, soil amended with biostimulant gave higher values of N_2-ase activity than compost only, this result explained the importance of the boost inocula added to the experimental soil. The highest significant values of N_2-ase activity at all determination periods were observed in rhizosphere amended with compost and humic acid in combination with biostimulant. This trend of results was observed in both tomato and pepper. This result may be due to the enhancement of humic acid to the native and introduced microorganisms and also increased the synergistic effect of inocula addition with other microorganisms. These results are in harmony with Meunchang *et al.* (2006) who mentioned that the compost promote plant growth when it amended with N_2 - fixing bacteria.

Table 4. Periodical changes in nitrogenase activity in moderately saline soil cultivated with tomato and pepper.

Treatments	Nitrogenase activity (µg C_2H_4/ hr./g dry soil) Periods (day) Initial	15	30	60	120
	Tomato				
Control	ND^{d}	8.09^{e}	25.68^{d}	57.69^{c}	69.69^{e}
Chemical fertilization	ND^{d}	6.06^{f}	9.48^{e}	40.42^{d}	78.4^{de}
Biostimulant	6.18^{c}	30.08^{cd}	34.87^{c}	58.01^{c}	81.0^{d}
Compost	7.08^{b}	33.41^{c}	35.00^{c}	69.2^{b}	77.2^{de}
Bio. + compost	6.18^{c}	102.2^{ab}	130.4^{b}	134.6^{ab}	106.6^{b}
Bio. + HA	6.04^{c}	33.03^{c}	34.20^{c}	68.2^{b}	98.2^{c}
Bio. + compost + HA	8.90^{a}	111.3^{a}	152.6^{a}	222.3^{a}	200.2^{a}
	Pepper				
Control	4.00^{d}	11.30^{e}	18.77^{e}	55.61^{f}	37.15^{e}
Chemical fertilization	4.02^{d}	12.41^{e}	27.70^{d}	58.90^{e}	33.13^{f}
Biostimulant	6.21^{c}	26.64^{d}	100.9^{b}	117.2^{b}	100.0^{b}
Compost	6.22^{c}	34.21^{c}	101.6^{b}	111.2^{bc}	99.87^{c}
Bio. + compost	8.38^{ab}	105.2^{b}	104.9^{b}	100.2^{c}	99.67^{c}
Bio. + HA	4.11^{d}	40.41^{c}	70.38^{c}	98.22^{d}	93.29^{d}
Bio. + compost + HA	11.02^{a}	179.4^{a}	127.7^{a}	144.6^{a}	136.7^{a}

Abbreviations as those stated in Table (2)

.Higher significant records of N_2-ase activity were observed in soil inoculated with biostimulant than soil treated with compost only, this result was observed at flowering and maturity stages (60 and 120 days) in pepper rhizosphere and at maturity stage in tomato rhizosphere. This result can be explicated by increasing the microbial activity in this stage where the beneficial root exudates are abundant. These results are in harmony with those obtained by Hanafy *et al.* (1998).

As a result of the boost inoculation with biostimulant during growth season, obtained data revealed that N_2-ase activity values were gradually increased through the first 60 days thereafter it decreased. This result explained the synergistic effect of continuous addition of inocula on survival and activities of beneficial N_2-fixers.

Oxidative enzymes

Oxidative enzymes (nitrate reductase, peroxidase and polyphenol oxidase) were estimated as a guide for plant tolerance of salinity. Data in Table (5) emphasized that the oxidative enzyme were affected with soil amendments.

Results clearly indicated that tomato cultivated in soil treated with chemical fertilizers gave the lowest values of plant oxidative enzymes, while the lowest values of these enzymes were observed in pepper plants cultivated in moderately saline soil without any amendments (control). In addition, tomato inoculation with biostimulant in combination with compost significantly increased the nitrate reductase, peroxidase and polyphenol oxidase activities compared to either biostimulant or compost each one individually. Similar trend of results was observed with tomato and pepper.

Table 5.Oxidative enzymes in tomato and pepper cultivated in moderately saline soil.

Treatments	Nitrate reductase activity as µ mol NO_2/	Peroxidase as absorbance/g fresh	Polyphenol oxidase as absorbance/g

	g dry leaves/hr.	leaves	fresh leaves
		Tomato	
Control	**58.90^{f}**	**1.659^{d}**	**0.245^{d}**
Chemical fertilization	**52.70^{g}**	**1.210^{e}**	**0.209^{e}**
Biostimulant	**99.00^{d}**	**3.422^{bc}**	**0.437^{c}**
Compost	**76.10^{e}**	**3.101^{c}**	**0.510^{bc}**
Bio. + compost	**141.3^{b}**	**4.342^{a}**	**0.519^{b}**
Bio. + HA	**100.5^{c}**	**3.831^{b}**	**0.224^{de}**
Bio. + compost + HA	**177.2^{a}**	**4.127^{ab}**	**0.585^{a}**
		Pepper	
Control	**107.4^{g}**	**1.011^{f}**	**0.111^{d}**
Chemical fertilization	**140.9^{f}**	**1.153^{e}**	**0.136^{c}**
Biostimulant	**155.2^{d}**	**2.224^{de}**	**0.351^{bc}**
Compost	**142.9^{e}**	**2.345^{d}**	**0.431^{b}**
Bio. + compost	**217.2^{c}**	**3.572^{b}**	**0.425^{a}**
Bio. + HA	**241.1^{b}**	**3.311^{c}**	**0.447^{ab}**
Bio. + compost + HA	**313.7^{a}**	**4.224^{a}**	**0.463^{a}**

Abbreviations as those stated in Table (2)

The highest values of the oxidative enzymes in pepper plants were observed in soil inoculated with biostimulant combined with compost and humic acid. This result may be due to the beneficial effect of the **native** microorganisms occurred in compost and their synergistic effect with biostimulant. These results are in agreement with Zhang *et al.* (2008) who stated that the positive effects of humic acid on plant growth could be due to mainly hormone like activities of the humic acid through their involvement in oxidative phosphorylation, protein synthesis, antioxidant and various enzymatic reactions.

CONCLUSION

In view of the obtained results it could be mentioned that the inoculation with salt-tolerant PGPR in combination with compost and humic acid increased the activity of microbial enzymes such as dehydrogenase, phosphatase and nitrogenase in rhizosphere. Also, the inoculation with PGPR increased the content of oxidative enzymes such as nitrate reductase, peroxidase and polyphenol oxidase and so improve plant defense against saline stress conditions.

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تأثير الريزوبكتريا المنشطة لنمو النبات والمتحملة للملوحة على نشاط بعض الإنزيمات الميكروبية وإنزيمات الاكسدة تحت الإجهاد الملحى

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أجريت هذه التجربة فى مزرعة كلية الزراعة بمشتهر عام 2011 تحت ظروف الصوبة بهدف دراسة تأثير تلقيح الطماطم والفلفل بالريزوبكتريا المشجعة لنمو النبات والمتحملة للملوحة على نشاط بعض الإنزيمات الميكروبية فى التربة مثل الديهيدروجينيز والفوسفاتيز والنيتروجينيز ، أيضاً دراسة تاثير التلقيح على نشاط بعض إنزيمات الأكسدة فى النبات مثل النيتريت ريدكتيز والبيروكسيديز والبولى فينول أوكسيديز. اتضح أن تلقيح التربة متوسطة الملوحة بالريزوبكتريا المتحملة للملوحة مع تدعيم التربة بالكمبوست بمعدل (10طن/الفدان) مع إضافة حامض الهيوميك بمعدل (4كجم/الفدان) كان له تأثير إيجابى على نشاط كل الإنزيمات تحت الدراسة مما إنعكس إيجابيا″ على نمو نباتات الطماطم والفلفل وذلك بالمقارنة مع النباتات المنزرعة فى التربة متوسطة الملوحة والغير مدعمة بأى إضافات (الكنترول).