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EFFECT OF COMBINATIONS OF BIOAGENTS WITH DIFFERENT ORGANIC MANURE ON CONTROLLING SEEDLING DAMPING OFF AND ROOT ROT DISEASES AND YIELD OF CUCUMBER PLANTS GROWN UNDER PLASTIC HOUSE

Gaafer,S.A.*; E. M. El-Sanafowy* and Maisa L. Abdel-El Moneim**
*** Veg. Res. Dep., Hort. Res. Inst., Agric. Res. Center, Egypt**
**** Central Lab of Organic Agriculture Research Center, Egypt**

ABSTRACT

Under clay soil texture of plastichouse conditions at Sakha Agric.Res. Station, the soil was treated with different microorganisms using in eight treatments. Cucumber was sown on 13 January, 2004 and 2005. Seedlings of 32 days old were transplanted into the soil of a plastichouse. The soil treatments were as follows: (1) control (CL). (2) Topsin (TP) 2.5 gm / L. (3) and (4) *Saccharomyces* composted in chicken manure (3) (YES + CHI) and in cattle manure (4) (YES + CM). (5) and (6) *Trichoderma harzianum*, composted in chicken manure (5) (TRI +CHI) and in cattle manure (6) (TRI + CM). (7) and (8) *Bacillus subtilis* composted in chicken manure (7) (BAC + CHI) and in cattle manure (8) (BAC+CM). The present study aimed to investigate the effect of the previous treatments on growth characters and fresh yield of cucumber and its components as well as pathogenicity study i.e. percentage of Pre, post, and survival plant. Each plant treatment received 100mL of it's proposed materials three times a week and the total applications were six. Accordingly, a complete randomized block was used with four replicates. The obtained results indicated that (TRI + CM) gave the highest plant length, the largest leaf area, the biggest fresh weight and number of leaves. At the same time, (TRI+CM) and (BAC + CM) led to increase significantly the plant leaf area index. Bacterial, (BAC + CM) and (BAC+CHI) resulted in the higher number and total yield per plant. In addition, significant increase of early yield was recorded by (BAC+CM), (BAC+CHI), (TRI+CM), (TRI+CHI) and (YES+CM) respectively compared with control.

The effectiviness of treatments on controlling damping off diseases for the host cucumber plant was ranged from 80 to 100%.

Bacillus subtilis (BAC) and *Trichoderma harzianum* (TRI) composted in cattle manure obtained the lower percent of disease incidence than the other treatments used including (control).

Keywords: Trichoderma harzianum, composted chicken manure, topsin, (Bac + CM), (TRI + CM), (YES + CH1).

INTRODUCTION

Two sets of selected microbial formula named compo MAX-1 (microbial inoculant contains microbial candidates of genera, *Thermoactinomcetes phanerochareta, Chaetomium* and *Trichoderma*) and Compo Max-2 (microbial inoculant bacterial and yeast strains). Compo Max – 1 and 2 had powerful capabilities of controlling soil born diseases (Abdel – Rahman and Sawan Omaima, 2003).

Bio organic Agriculture aims to protect the natural balance, therefore it has become a necessity in greenhouses, whereas chemicals are used intensively (Tuzel *et al.*, 2001).

In cucumber experiments, Chigaleichik *et al.* (1999) treated the greenhouse with microorganisms before planting cucumbers, and they noticed the root systems was stimulated and developed very well. In addition, Kioko *et al.* (1998) treated the soil by *Bacillus sp.* and they found that the root significantly protected from the soil borne diseases through the colonized bacteria by bacillus. The same figure was observed by Wenshi and Wu (1998), they worked on *Bacillus sp.* and *Trichoderma sp*, they added that, plant dry weights significantly increased when the media treated with *Bacillus* and *Trichoderma spp.*.

Maisa (2004) treated the greenhouse cucumber seedlings with *Bacillus spp.* and *Bacillus subtilis* before transplanting. She found that damping off and root rot soil borne diseases not exceeded 1.00% in treated plant. Also, dry matter and chlorophyll A and B significantly increased.

Fifteen antagonistic bacteria were isolated by Hessenmuller and Zeller (1996). Four isolates only reduced strawberry root disease. The greatest inhibitory activity was found by *Bacillus licheniformis*. Whearis, *fragariae*, *F. oxysporum* diseases were controlled by *Bacillus subtilis* in strawberries crop. (Okayama *et al.*, 1991). Number of leaves buds, fruits, total weight of fruits and average fruit weight were increased when Wange *et al.* (1997) treated strawberry roots with microbial inoculation and used biofertilizers.

Abou Hussein *et al.* (2002) and b] studied the effect of compost i.e. chicken manure (dry manure of extract from chicken after 48h.) and compost (40m^3 and 60 m^3/fed.) with two kinds of biofertilizers added to the soil (Suspension from yeast, Pseudomonas and phosphate dissolving bacteria or inoculated with potato tuber. They found that plant height, number of leaves, leaves fresh and dry weight, stem fresh and dry weight were significantly increased with chicken manure + biofertilizer treatment. Also, total yield and mineral nitrate content in tubers was significantly increased.

In cosmos crop, Safwat (2004) found that biofertilizer mixture of (*Azotobacter choococcum*, *Azospirillum lipoferum*, *Bacillus polymixa*, *Bacillus megatherium* and *Pseudomonas fluorecence*) with different rates of macro elements increased vegetative growth (Plant height, stem diameter, fresh and dry weights of shoots and number of branches / plant) compared with macro elements alone.

ELKholi *et al.* (2004) compared, *Azospirillum brasilence*, *Azoto-bacter chroococcum* and *Bacillus megatherium* under different levels of nitrogen. They found that biofertilizer had significant effect on dry weight of leaves, total soluble solid and total yield of sugar Beet. Whereas, El Etr *et al.* (2004) stated out that, Nitrogen, phosphorus and potassium uptake were increased significantly when compost inoculated. They also found that plant dry weights and yield were increased in pea and wheat plants.

The aim of this work was to study the ability of organic amendments and the beneficial of microorganisms to control soil borne diseases and increase yield and fruit quality of cucumber plants.

MATERIALS AND METHODS

General cultural practices:

The trial was carried out in a walk–through plastichouse Gable structure with tewin shape. The dimension of the plastichouse unit was 55 meters long, 16 meters wide and 3.5 meters high. The plastichouse installed at sakha Agric. Res. Station, Kafer EL-Sheikh Governorate. Speedling Trays were filled with mixture of peat–moss and vermiculite (1:1) v/v. Cucumber (*Cucumis sativus* L.) Delta star F_1 hybrid seeds were sown on 13 January, 2004 and 2005 respectively. Seedlings of 32 days old were transplanted under unheated plastichouse. The plastichouse had ten raised beds for cultivation each was 100cm wide, 20 cm high, double rows have been planted on each bed at a distance of 50 cm between rows and 50 cm between plants. Beds were supplied with four tons of compost. Drip irrigation system was used and the other cultural practices were applied as recommended by Ministry of Agriculture. Plants were supported vertically by using plastic strips. Soil physical and chemical properties were tabulated in Table (1).

Plant treatments:

Eight treatments were conducted when cucumber seedlings were transplanted namely:

1- Control (CL)

The plant received only 100 ML of irrigation water.

Table (1): Physical and chemical properties of the plastic house soil before conducting the experiments in (2004 and 2005) at sakha.

	1st season	2nd season
Physical analysis		
Sand %	33.40	32.40
Silt %	28.20	29.80
Clay %	38.40	37.80
Field capacity %	38.00	39.00
Total Ca Ca_3 %	2.00	1.70
Bulk density g/cm^3	1.10	1.18
Texture class	Clay	Clay
Chemical analysis		
PH in 1: 2.5 soil / water suspension	7.9	7.60
E.cm mbos/cm at 25°C in 1:5 soil water extract	4.37	3.42
Organic matter %	3.11	2.86

2- Topsin (TP)

Topsin M70% wp, at a concentration of 2.5 grams was diluted in one liter and each plant received 100ML.

Preparation of the biological agents:

Isolation of microorganisms from organic manure were done by inserting one gram from cattle manure and chicken manure in bottels (600mL) contained 99mL of sterile distilled water. Bottles were shaked on electric shaker for two hours. Diluted suspensions were prepared as follows:-

One mL of suspension after a vigorous agitation was drawn and added to 99 mL of sterile water in plugged flask, this procedure was repeated to obtain serial dilutions 10^{-4}, 10^{-5} and 10^{-6}. Media used to isolate different groups of microorganisms, and dilutions were used for isolation are presented in Table (2).

Table (2): The microorganisms which was isolated from different organic manures.

Organic Manure	Fungi		Bacteria		Actinomycetes	
	Identifica-tion	No. of colonies	Identifica-tion	No. of colonies	Identifica-tion	No. of colonies
attle manure	*Trichoderma sp.*	2×10^{-4}	*Bacillus sp.*	2×10^{-5}	-	-
Chiken manure	*Trichodermasp.*	2×10^{-4}	*Bacillus sp.*	6×10^{-6}	*Streptoomyces sp*	1×10^{-5}

Different isolated microorganisms were grouped and stored on (NGA) slant (Dowson 1957).

Treatments preparations:

Treatments (3) and (4) prepared as follows:-

Saccharomyces composted in chicken manure (YES + CHI) (3) and composted also in cattle manure (YES + CM) (4) were grown in Glucose peptone-yeast extract medium (Papavizes and Davey, 1959) for one week and CFU were adjusted to be 30×10^{-6} mL.

Treatments (5) and (6) preparated as follows:-

The fungus *Trichoderma harzianum* composted in Chicken manure (TRI + CHI) (5) and cattle manure (TRI + CM) (3) separately and grown in Gliotoxin fermentation media for 9 days and adjusted to be 30×10^{-6} mL. according to Abdel moity and Shatla (1981).

Treatments (7) and (8) preparated as follows:-

The bacterium *Bacillus subtilis* composted in chiken manure (7) (Bac + CHI) and cattle manure (8) (BAC + CM) separately grown in nutrient Glucose broth for 48hrs. The bacterial suspension was adjusted to be 30×10^{-6} cells /mL.

Each cucumber plant received 100mL of the tested liquied material according to the work plan (three times a week) and the total applications were six times.

Measurements:

1- Vegetative growth parameters.

Plant height cm, Number of leaves per plant, leaf area cm^2/ Plant, leaf area index /plant and the number of branches / plant.

2- Leaves fresh weight (gm)/ plant.

3- Yield and its compoments which contained:

- Early yield (kg/ plant) for the first four pickings.
- Total fruit number / plant.
- Total yield (kg/ plant).
- Average fruit length (cm), diameter (cm) and fruit shape index.

4- Chemical composition.

- Dry weight of leaves (gm) and % of fruits.

- Soluble solids content (SSC).
- Total chlorophyll content measured using Minolta chlorophyll Meter spade - 501.

5- Pathogenic studies (Damping - off and root rot incidence) .

Isolation of the causal organisms:

Samples of cucumber plants showing identical symptoms of root rot and damping off were collected washed, dried and sterilized. Surface sterilized plant materials were dried between two sterilized filter papers. The plant materials were cut into small pieces with sterilized scalpel and then cultured on plane agar medium. Plates cultured were incubated at (25-28)°C. Plates were examined periodically and the appearance mycelia were transfered to potato dextrose agar (PDA) medium. The purified fungi were transfered to slant of nutrient glucose agar (NGA) medium and inoculated at 28°C for 7 days. The fungal culture kept at 5 °C for further studies.

Identification:

Isolated fungi were identified according to their cultural properties and morphological characteristics described by Gilman 1957, Barnett 1960 and Singh 1982.

Pathogenicity test:

Inocula of the two isolates of each identified pathogen (*R. Solani, F. solani* and *Sclerotiom rolfsii*) were prepared using the method mentioned by Abd Elmoity (1985).

Determinations:

- Percentage of pre emergence was determined after 5, 7 and 9 days from planting date.
- Percentage of post emergence was determined after 9, 11 and 15 days from transplanting date.
- Percentages of survived plants were determined after one month from transplanting.

Accordingly, eight treatments were designed in a complete randomized blocks with four replicates. Five plants from each treatment were labeled for measurement of vegetative growth parameters and yields. All data were subjected to statistical analysis of variance according to Gomez and Gomez (1984) using L.S.D. at 5% level.

RESULTS AND DISCUSSION

Data in Table (3) presented the effect of studied treatments on plant height cm, leaf area cm^2, leaf area index %, number of shootper plant and leaves fresh weight per plant (gm) during the two seasons. The data cleared that, *B. subtilis* (BAC), *T. harzianum* (TRI), and *saccharomyces sp.* (YES) composted in cattle and chicken manures had favourable effects on plant (height leaf area, number of leaves) and leaves fresh weight. This favorable effect reached to the significant level compared with topsin and control treatments. In addition, *Bacillus subtilis* (BAC + CM) composted in cattle manure. cattle compost media for *T. harzianum* (TRI + CM) and *B. subtilis* (BAC + CM) led to significant increases of plant leaf area index in both years of study as shown in Table (3).

Table (3): Vegetative growth and leaves fresh weight parameters of cucumber (*Cucumis sativus* L.) as affected by different soil treatments in 2004 (A) and 2005 (B) seasons A.R.C. Sakha.

Measurements / Treatments	Plant height (cm)		Plant leaf area (cm^2)		Plant leaf area index %		No. of leaves / Plant		No. of branches / Plant		Leaves fresh weight / plant (gm)	
	A	B	A	B	A	B	A	B	A	B	A	B
Control CL	212.0	206.0	210.4	203.2	5.3	5.2	37.0	31.0	2.3	2.7	405.3	377.8
Topsin TP	219.3	217.3	226.8	210.6	5.7	5.3	39.0	34.0	2.3	2.7	505.8	446.0
YES + CHI	240.3	230.7	245.1	229.4	6.1	5.7	45.0	36.7	2.7	2.7	527.3	480.0
YES + CM	242.3	237.3	241.7	233.3	6.3	6.0	45.3	41.0	3.3	2.7	535.3	487.0
TRI + CHI	254.8	237.7	247.6	235.5	6.5	5.9	47.2	41.0	3.4	2.6	593.0	500.8
TRI + CM	357.3	234.0	252.0	236.1	6.0	6.2	47.0	42.0	3.4	2.3	610.0	469.8
BAC + CHI	257.3	245.3	259.2	243.2	6.5	6.3	49.1	44.2	3.4	2.7	635.5	516.8
BAC + CM	262.3	252.3	262.3	251.1	7.3	6.2	49.3	46.7	4.3	2.3	640.0	576.8
LSD at 5%	26.2	18.4	26.1	17.7	1.3	0.7	5.8	3.5	1.3	N.S.	142.6	86.2

Table (4): Fruit yield and its components of cucumber (*Cucumis sativus* L.) as affected by different soil treatments in 2004 (A) and 2005 (B) seasons A.R.C. Sakha.

Measurements / Treatments	Total fruit numbers / plant		Early yield kg / plant		Total yield kg / plant		Average fruit length (cm)		Average fruit diameter (cm)		Fruit shape index	
	A	B	A	B	A	B	A	B	A	B	A	B
Control CL	32.3	28.0	0.5	0.5	3.2	2.7	14.6	14.0	3.2	3.2	4.5	4.3
Topsin TP	31.2	30.7	0.6	0.5	3.5	2.8	15.9	15.0	3.3	3.2	4.7	4.8
YES + CHI	37.0	30.6	0.7	0.5	3.6	3.0	16.7	16.6	3.6	3.2	4.6	5.1
YES + CM	37.0	30.2	0.7	0.6	4.0	3.1	16.9	16.8	3.7	3.4	4.8	5.0
TRI + CHI	36.9	33.4	0.7	0.6	4.4	3.3	18.2	16.9	3.7	3.3	4.8	5.0
TRI + CM	39.4	34.2	0.8	0.6	4.4	3.5	16.7	17.6	3.7	3.3	4.7	4.9
BAC + CHI	40.2	36.1	0.8	0.7	4.6	3.5	18.0	17.2	3.8	3.3	4.8	5.2
BAC + CM	41.5	35.5	0.9	0.8	4.7	3.8	18.2	17.2	3.8	3.5	4.9	5.1
LSD at 5%	4.1	3.5	0.1	0.1	0.9	0.6	1.9	1.4	0.2	0.2	0.3	0.7

While, *T. harzianum* and *B. subtilis* composted in chiken manure reached the level of significance in the second year only compared with control. Considering the number of branches per plant the data were inconsistent in both studied years. These data agreed with the findings of chigalichik *et al.* (1999), Kioko *et al.* (1998) through their discussion about the root development with biofertilizer. Safwat (2004) came to conclude the beneficial effect of biofertilizer in growth and development of cosmos plant.

Data tabulated in Table (4) show the influence of the studied treatments on cucumber (*Cucumis sativus* L.) yield and its components. Superiority of *Bacillus* composted in cattle manure (BAC+CM) and *B. subtilis* composted in chicken manure (BAC + CHI) gave significantly higher total fruit number per plant followed by fungus *T. harzianum* composted in cattle manure (TRI+CM) and *T. harzianum* composted in chiken manure (TRI+CHI) compared with other studied treatments and control in 2004 and 2005. Similar results were found for total yield Kg/ plant.

Regarding early yield in Table (5), data show that bacterial (BAC+CM), (BAC+CHI), Fungial (TRI+CM), (TRI+CHI) and *actinomycetal* (YES+CM) resulted in higher early yield than those of other treatments in the two years. In addition *Bacillus subllis* composted in cattle manure (BAC + CM) had the highest early yield in both seasons. Such data were confirmed proviously by Wange *et al.* (1997) and Abou Hussein *et al.* (2002) through this discussion on strawberry and potato tuber respectively. With respect to the average fruit length, all the studied treatments i.e. (YES+CHI), (YES+Cm), (TRI+CHI), (TRI+CM), (BAC+ CHI) and (BAC+CM) gave significantly longer fruits than the control in both years.

Table (5): leaves and fruits dry weights, soluble solid content (SSC), Total Chlorophyll of cucumber (*Cucumis sativua* L.) as affected by different soil treatments in 2004 (A) and 2005 (B) seasons.

Measurements / Treatments	Leaves dry weight / plant (gm)		Fruit dry weight %		Soluble solids content (SSC)		Total chlorophyll %	
	A	B	A	B	A	B	A	B
Control CL	56.4	50.5	4.3	4.4	4.0	4.2	39.5	40.9
Topsin TP	62.3	57.2	4.4	4.4	4.6	4.1	47.3	31.4
YES + CHI	47.7	63.2	4.3	4.3	4.4	3.7	49.7	39.7
YES + CM	73.9	68.5	4.5	4.7	4.4	4.5	49.8	41.3
TRI + CHI	81.9	70.0	4.6	4.4	4.5	4.3	49.9	41.4
TRI + CM	88.3	70.0	4.6	4.4	4.6	4.4	50.1	45.0
BAC + CHI	89.7	72.7	4.6	4.2	4.5	4.6	49.8	45.2
BAC + CM	92.7	75.0	4.7	4.6	4.6	4.6	52.2	49.3
LSD at 5%	19.3	19.4	0.4	N.S.	N.S.	N.S.	6.4	N.S.

Meanwhile, *B. subtilis* composted in cattle manure (BAC+ CM) gave *Saccharomyces* spp. composted in cattle manure (YES+CM) only resulted in bigger fruit diameter. The data expressed also that Bacterial (BAC+CM),

(BAC+CHI), Fungal (TRI+CM) and *actenomycetal* (YES + CM) induced significant fruit shape index % than control.

The data of cucumber chemical analysis are tabulated in Table (5) for the (A) and (B) seasons. Cucumber plant leaves dry weight was significantly weighed with (BAC+CM), (BAC+CHI), (TRI +CM) and (TRI+CHI) treatments compared with control. While, there was no significant effect due to the treatments on soluble solids content (SSC) in both years. The percentage of fruit dry weight and the total chlorophyll inconsistent in both studied years. The data did not agree with those of Maisa (2004) concerning the total chlorophyll may be due to the different seasons and in turn different environmental conditions.

The data in Table (6) show the isolation and identification of the causal organisms. The effect of different isolates were No_1, No_2 of *R. solani* and No_2 of *S. rolfsii* wheares F. solani isolate No_1 was the least destructive one. This rapid and severe damage caused by *S. rolfsii* or *R. solani* might be due to the synergistic action between polyglocturonase and oxalic acid produced by pathogenic *S. rolfsii* as mentioned by Bateman and Beer (1964).

Table (6): Pathogenicilly test of *Rhizoctonia solani*, *Fusarium solani* and *Sclerotium rolfsii* measured as pre and post emergence damping off as well as healthy survival of cucumber plants.

Different pathogenic isolates	% pre	% post	Survival plant
Rhizoctonia solani $_1$	60	40	0.0
Rhizoctonia solani $_2$	50	50	0.0
Fusarium solani $_1$	50	30	20
Fusarium solani $_2$	60	30	10
Sclerotium rolfsii $_1$	70	15	15
Sclerotium rolfsii $_2$	80	20	00
Control	0.0	0.0	100
LSD at 5%	3.97	6.97	4.44

The tabulated data in Table (7) illustrated the combination between cattle and chicken manures as media for different bio agents for controlling root rot and damping off diseases. Both *B. subtilis* and *T. harzianum* composted in cattle manure had significantly higher percent of disease incidence than the chemical treatment and standard. At the same time, (BAC+CHI) and/or [(TRI +CHI) and (YES+CM) and (YES +CHI) came to reach to the significant level. The data were in line with those of Maisa (2004), Abdel- Rahman *et al.* (2003) and Hessenmuller and Zeller (1996).

Table (7) Effect of different combination between organic matter and bio agents on the controlling of root rot and dumping off diseases in cucumber plants under plastichouse condition.

Treatments / Measurements	Control CL	Topsin TP	YES + CHL	YES + CM	TRI + CHI	TRI + CM	BAC + CHI	BAS + CM	LSD at 5%
% of disease incidence	100	33.3	6	5	5	3	4	3	1.37
% of efficiency of treatment	0.0	66.7	94	95	95	97	96	97	1.37

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تأثير استخدام الكائنات الحيوية مع مستخلصات المخلفات العضوية لمقاومة سقوط البادرات واعفان الجذور على زيادة الصفات المحصولية لنباتات الخيار تحت ظروف الصوب البلاستيكية

سامى عبد الجواد جعفر* ، الصنفاوى محمد الصنفارى* و مايسه لطفى عبد المنعم**

* أقسام بحوث الخضر – معهد بحوث البساتين – مركز البحوث الزراعية – مصر

** معهد بحوث أمراض النباتات – مركز البحوث الزراعية – القاهرة

تحت ظروف التربة الطينية فى الزراعة المحمية بمحطة بحوث سخا تمت معاملة الصوبة بثمانى معاملات. تم زراعة بذور الخيار فى ١٣ يناير ٢٠٠٤ للموسم الأول ونفس التاريخ للموسم الثانى. شتلات الخيار بعمر ٣٢ يوم تمت زراعتها فى الصوب ذات الجور المعاملة بثمانى معاملات كالتالى:-

١- الكنترول
٢- المبيد توبسن (فطرى جهازى) ٢,٥جم / لتر
٣- سيكارميستس نمت على سبلة دواجن
٤- سيكاروميستس نمت على سبلة مواشى
٥- ترايكوديرمانارزيانم نمت على سبلة دواجن
٦- ترايكوديرما نمت على سبلة مواشى
٧- باسلس هارزيانم نمت على سبلة دواجن
٨- باسلس سبتيلس نمت على سبلة مواشى

فى تجربة صممت قطاعات كاملة العشوائية بحيث تتال كل معاملة ٦ مرات من المحلول فى أسبوعين وكان الهدف من هذه الدراسة توضيح تأثير المعاملات على النمو الخضرى والمحصول الكلى والمبكر ومدى مقاومة النباتات لأمراض الذبول. وقد أوضحت النتائج أن المعاملة رقم (٦) قد أعطت أفضل النتائج على الإطلاق فى طول النبات ، وأكبر مساحة أوراق، وأكبر وزن وعدد الأوراق لنبات الخيار. وفى نفس الوقت المعاملة (٦)، المعاملة (٨) قد أعطت معنوية فى دليل مساحة الورقة للنبات. وأن المعاملة (٨) والمعاملة (٧) قد أعطوا أكبر محصول للثمار عددا ووزنا للنبات. كما أن المحصول المبكر كان من نصيب المعاملات (٨)، (٧) ، (٦)، (٥)، (٤) على التوالى.

من الناحية المرضية لقد وجد أن المعاملة (٨) والمعاملة (٦) كانوا أكثر المعاملات لمقاومة أعفان الجذور وموت البادرات حيث سجلوا أقل نسبة مئوية للإصابة بأمراض أعفان الجذور وموت البادرات المختلفة.