BIOLOGICAL CONTROL OF POWDERY MILDEW DISEASE ON MARIGOLD (*Calendula officinalis* L.) AT FAYOUM

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ABSTRACT

Marigold (*Calendula officinalis* L.) is one of the important economically crops, which belongs to family Astraceae. Marigold represents one of the most important and economic ornamental crops in the Arab Republic of Egypt. The economic importance of this crop appears in both local consumption and exportation purposes. Cultivated area of marigold in 2005 growing season reached about 550 Fadden in open fields, which yielded 9397 ton flowers. Several disease attacked marigold plants in all growing stages as powdery mildew. The Present work was designed to solve a part of this dilemma and control this disease by non-chemical methods.

The present investigation aimed at studying the effect of bio control agents in controlling marigold powdery mildew.

Different microorganisms were isolated from phylloplane of healthy marigold plant and used as antagonists against different pathogens. *Trichoderma harziamum* and *Bacillus subtilis* were the most effective antagonists against all tested pathogens as well as increasing the average number of flowers/plant and average weight of flowers/plant One week interval was the best time for using both organisms as antagonists.

Key words: Disease, Biological Control, Powdery Mildew, Marigold.

INTRODUCTION

Marigold (*Calendula officinalis* L.) is considered one of popular the flowering annual plant, belongs to family Asteraceae, native to canary Island and Iran. It is a small herb and The height of the plant ranges between 40 and 60 cm., leaves are simple and alternate flowers are in heads with yellow or orange rays. The plant is used as an ornamental flowering plant and flower heads are sometimes used in cookery to flavor soups and stews. In medicine, the plant is used as a vulnerary and anti-emetic, **(Bailey, 1978).** On the other hand, marigold flowers are richen carotenoids specially beta carotene which can be used in food processing to retain the orange natural color and as a supplement for vitamin A precursor so, it can be added to butter, margarine, beverages, ice cream, beverage powder, yoghurt and soups as a coloring agent. **(Consell and Knewstubb, 1983).**

According to the Agricultural Economic & Statistical Department, Ministry of Agriculture and Land Reclamation, the cultivated area of marigold in 2005 growing season reached 550 Feddans as open fields, yielded 9397 tons of flowers .

Powdery mildew is among the most effective diseases causing considerable losses to marigold production **(Pyeott &Kretchun, 1983** and **Alfieri *et al.*,**

1984). The disease can be easily recognized by the white lesions spreading or covering the most of the plant foliage specially leaves.

The disease can be caused by the obligate parasites *Erysiphe pologoni, Sphaerotheca fuliginea* and *Erysiphe cichoracearum,* which absorb their foods by the haustoria from the epidermal cells also, it characterized by the white powdery lesions during the growing season and by the brown – black lish ascocarps at the

The present study aimed to study the effect of bio control agents in controlling marigold powdery mildew

MATERIALS AND METHODS

1-Survey of marigold powdery mildew disease at Fayoum governorate:-

Surveys were conducted from early October to mid December during 2004 and again from late January to mid April 2005 in different localities of Abshway, Yousoff Alsadik, El-Fayoum to assess the incidence and severity of powdery mildew on marigold and to establish the identity of the causal organism. In each locality ten to twenty field plots samples consisting of aerial parts of the plant (leaves and stems) was collected from the infected marigold plants. Incidence of the disease (percent occurrence) on each locality was calculated as percent according the method given by **Johnston and Both (1983)** as follows:

$$\text{Disease Incidence \%} = \frac{\text{Number of infected plant units}}{\text{Total number (healthy and infected) of units assessed}} \times 100$$

Disease severity and disease index:-

Each grade in this scale represents a numerical rating proportional to the severity of disease at a particular time. 0.0. Healthy. no trace of infection, at 25 slight infection, fine coating of powdery growth on upper leaves, stem free from powdery Mass.50 moderate infection, nearly 50% plant is covered with powdery mass stem also infected, 75 Nearly 55% leaves infected whole plant is covered with powdery mass. Stem also infected 100 All the leaves as well as stem and other parts coated with thick powdery mass. Leaf co lour changes to grayish and starts drying.

$$\text{\% Disease severity} = \frac{\Sigma\, n.v}{N.V} \times 100$$

Where:

n=Number of infected leaves in each grade category .
v=Numerical value in each grade category .
N=Total number of leaves on the plant .
V=The highest degree of infection (i.e.,4) ..

Media preparation:

a. Peptone Dextrose Agar plus Rose Bengal and Streptomycin

KH_2Po_4 -1.0 g. $MgSo_2$ - 7 H_2O 0.5 g, peptone 5.0 g. Dextrose 10.0 g. Rose Bengal 0.001g, streptomycin 30.0mg /L. Distilled water 1000 ml and agar 20.g, sterilized at/atm. 121°C for 30 min and P.H was adjusted to 5-5.2 **(Johnson *et al* ., 1960).**

b. Soil extract agar.

Soil extracts 1000 ml, $K_2 HPO_4$ 0.2 g, agar 20.0 g, sterilized at 1.5 atm.128°C. for 20 min and the PH was adjusted at 7.2 **(Lochhead, 1940)**

Healthy marigold leaves were collected from El-tonsy farm among the highly infested plants with powdery mildew One gram of each leaves. samples were placed in bottle (600 ml in capacity) and contains only. 99 ml of sterilized distilled water. Bottles contain leaf samples plus Water were shacked for 2 hours. Serial dilutions of 10^{-4}, 10^{-6} were prepared from the original by adding 1 ml to 99 ml of sterilized distilled water. Different groups of Petri dishes each contain one of aforementioned media were used to isolate philosphere Microorganisms. Petri dishes were inoculated using one ml of dilulton 10^{-4},10^{-6} of the suspensions of each leaf samples, respectively. Inoculated plates were then incubated at 28°C Plates were examined periodically. Developed colonies were transferred, purified and only –non- pathogenic isolates were selected for further studies

Identification :-

***Trichoderma* species**

The isolated *Trichoderma* species were identified according to their. Cultural properties and Microscopic characteristics according to **Rifai (1969).** Identification was Kindly carried out in Mycology and plant Disease survey Research, plant pathology institute, ARC, Egypt.

***Bacillus* spp:**

Bacterial isolate was examined Microscopically and proved to be of Gram positive, rod shaped cells, shorten rod, motile in nutrient Perth medium, endospore formers. aerobic, cat ales positive and produce acid from glucose. These were considered as *Bacillus* spp. as species of genus

Bacillus and given different code numbers. to determine the *Bacillus* species the isolates wear exposed to further tests as described in (**Bergys manual of Determinative Bacteriology 1994).**

Biological control studies

preparation bio control agents:

The following two different of bio preparatign of Bioagents were used in these studies

1- *Trichoderma harzianum*
2- *Bacillus subtilis*

Trichoderma harzianum was grown separately on liquid gliotoxin Fermentation Medium **(Brian and Hemming 1945)** under complete darkness condition, Just to stimulate secondary metabolites production **(Abd El-Moity and shatla 1981)** After 9 days cultures were prepared as suspension and number of prop gules was adjusted in all isolates to be 32 $\times 10^6$ prop gules / ml – using Hemolytic meter (*Bacillus subtilis* isolate) was grown on nutrient glucose broth (NGB) **(schaad 1980)** After 3 days number of Bacterial cells in each culture isolate was adjusted to be 32×10^6 c.f.u /ml. All antagonistic preparations were used at the different rate of /100 liter of water Adhesive material "auperfilm" was added at the rate of /3 ml /1 liter of the diluted culture suspension

Formulation of bio agents:

Different bio control agents were formulated as spore suspension using method developed by **Abd El-Moity (1985).** Prepared suspensions were adjusted to be contain 3×10^6 cfu/1 ml.

EXPERIMENTAL RESULTS

Survey of marigold powdery mildew disease at Fayoum Governorte during 2004 -2005 growing season:

Data in table (1) represent the disease incidence percentage and disease severity of Marigold powdery mildew during seasons 2004-2005. Regarding the disease incidence Etsa center showed the highest percentage of disease incidence and the percentage were reader 0,40, 50, 70 and 100 % during months October, December, January, March, and April respectively, whereas the lowest ones, 0, 20, 30, 50, and 70%, were registered at Yossef El - Sedik during the same months.

Regarding disease severity, Sennoris center show the highest percentage disease incidence 0, 62.5, 56.25, 62.5, and 76.39 % during months October, December, January and April. Whereas Abshaway center gave the lowest percentage and only 0, 55.5, 56, 66.43 and 62.5% were recorded during the same months

Table (1):Survey of marigold powdery mildew disease incidence at Fayoum Governorate during 2004 growing season.

County	% Disease incidence					Disease severity				
	October	December	January	March	April	October	December	January	March	April
Abshaway	0	30	50	70	100	0	55.5	56	66.43	62.5
Yoseff El sedeak	0	20	30	50	70	0	57.5	61.11	60.00	68.14
El Fayoum	0	20	40	60	80	0	41.67	50.00	58.49	68.75
Etsa	0	40	50	70	100	0	58.33	50.00	65.23	67.5
Sennoris	0	20	60	80	90	0	62.5	56.25	62.5	76.39
L.S.D at 5%		3.2	5.4	7.6	10.1		6.5	6.3	N.S.	7.9

Isolation of antagonistic microorganisms from phyloplane

Obtained from the experiment conducted to isolate microorganisms from healthy marigold philosopher indicated that, *Trichoderma harzianum* and *Bacillus subtilis* were frequently isolated.

Table (2) : The isolated microorganisms from philosophere

Source	Fungi %				Bacteria %		
	T.harzianum	*Aspregillus* s.p	*Rhizopus* s.p	*Penicillium* s.p	*B.subtilis*	*Pseudo-Monas* sp.	Others
Etsa	36	32	25	15	45	23	15
Sennoris	43	12	22	11	53	13	23
Abshaway	51	10	7	8	62	36	17
E-lFayoum	61	11	21	10	33	23	12
Yoseff El sedeak	73	17	23	7	47	31	16

Biological control studies

Effect of interval period between two sprays of different biological preparation on marigold powdery mildew disease incidence

Effect of period between two applications on the efficacy of used antagonist was studied. Different antagonists were sprayed weekly, every two weeks or every three weeks. obtained data are presented in Table (3). Presented data indicate that, all antagonists behaved as each others and negative correlation between length of interval period and efficacy of the antagonist was observed. All antagonists, showed the highest effect when treatment was repeated every week. The effect was reduced when interval period was increased to be two or three weeks. *B. subtilis* and *Trichoderma hrarzianum* were the most effective bioagents when marigold plants were received one of these antagonists, every week. The lowest powdery mildew disease severity Regarding yield in different treated plants, data obtained indicated that, all treatments were significantly increased yield of marigold. Clear negative relation was observed between disease severity and yield but not with percentage of disease incidence.

Table (3): Effect of interval period between two sprays of different biological preparation on marigold powdery mildew disease incidence.

Treatments microorganisms	Interval period	% of Disease	Disease Severity	Average number flowers / plant	Average weight of flowers(g)plant
T. harzianum	1	25.00	28.08	159.00	3084
	2	45.00	30.63	156.00	1981
	3	52.50	45.09	145.00	1725
Mean		**40.83**	**34.6**	**153.33**	**2136.67**
B. subtilis	1	20.00	23.38	160.00	2704
	2	36.25	36.38	147.00	2190
	3	47.50	50.92	134.00	1541
Mean		**34.58**	**36.89**	**147.00**	**2145**
Control		100.00	78.36	108.00	756
L.S.D For microorganisms (A) at 5%		**N.S.**	**N.S.**	2.48	9.59
L.S.D For concentrations (B) at 5%		10.62	12.05	3.04	11.73
L.S.D For A×B at 5%		**N.S**	**N.S**	4.30	16.62

Effect of different concentrations of Bioagents on marigold powdery mildew disease incidence

Effect of different concentrations of bioagents on controlling marigold powdery mildew disease incidences was also studied. The aim of this experiment was to determine the effective concentration be used for each antagonist protect marigold agonist powdery mildew. Data in Table (4) reveal that, spraying marigold plants with *T. harzianum* or *B.subtilis* at any concentration led to significantreduction in the percentage of disease incidenceand disease severity. Reduction in disease incidence was correlated with increasing the number and weight of flowers /plant. Data also show that in general *B subtilis* was more effective than *T.harzianum* in controlling powdery mildew at concentration (2 %) .

Table (4) Effect of different concentrations of bioagents on marigold powdery mildew disease incidences

Treatments	concentrations	% of Disease	Disease severity	Average number flowers/plant	Average weight of flowers(g) / plant
T. harzianum	20	10.00	10.07	170.00	4063
	10	17.00	16.77	163.00	2771
	5	37.00	62.85	145.00	2247
Mean		**21.33**	**29.90**	**159.33**	**3018**
B. subtilis	20	7.00	6.38	160.00	3088
	10	15.00	18.22	161.00	2415
	5	42.00	38.85	148.00	1968
Mean		**21.33**	**21.15**	**156.33**	**2490.33**
Control		100.00	82.01	110.00	770
L.S.D For microorganisms (A) at 5%		N.S.	5.44	N.S.	5.83
L.S.D For concentrations (B) at 5%		8.25	6.66	3.31	7.15
L.S.D For A×B at 5%		N.S	9.42	4.68	10.11

DISCUSSION

Recently, man realized that, using highly toxic substances in agricultural led to great disturbance in biological balance. This disturbance led to the appearance of new pests and cause reduction in number of natural enemies and increase the accumulated toxic chemicals in human food chain. In 1991, the law of organic agriculture was issued in European Community under the number 2092/91 **(Manon, 1998).** New group of food products was appeared in the markets under different names i.e. organic, biodynamic and ecological food. All these names indicated that, no synthetic chemicals were added during production or processing. The present work was designed to reduce using toxic chemicals in agriculture process and find out the most suitable non-chemical and natural chemical methods to protect marigold plants against powdery mildew disease

Biological control using antagonistic bacteria has been reported as an attractive alternative due to their ability to antagonize the pathogen by different modes of action and to effectively colonize distinct plant habitats **(Raaijmakers *et al.* 2002).** Most attention has focused on the use of Gram-negative bacteria belonging to genera *Pseudomonas* or *Erwinia* **(Braun-Kiewnick *et al.*, 2000; Cartwright *et al.*, 1995; Costa *et al.*, 2001; Slininger *et al.*, 2000).** Gram-positive *Bacillus*, however, possess several advantages that make them good candidates for use as biological control agents (BCA). First, their antagonestic effect is caused by their ability to produce different types of antimicrobial compounds such as antibiotics (e.g., bacilysin. Iturin, mycosubtilin) and siderophores **(Shoda, 2000).** Second, they are able to induce growth and defense responses in the host plant **(Raupach and Kloepper, 1998).** Furthermore, *Bacillus* is able to produce spores resistant to UV light, which allows them to resist adverse environmental conditions, and permits easy formulation for commercial purposes **(Raaijmakers *et al.*, 2002).**

Reaction of different antagonists against powdery mildew was studied. Data obtained indicated that *T. harzianum* show good effect against the

powdery mildew. This effect in antagonism might be due to that *Trichoderma* sp. act through different mechanisms, i.e. mycoparasitism **(Abd El-Moity and Shatla, 1981 Martin and Hancock, 1987 and Benhamoud and Chet, 1993).** Also, through production of anti fungal substances **(Turner, 1971, and Hayes, 1992),** *Trichoderma* sp. also act through production of destructive enzymes i.e. chitenase **(Abd El-Moity, 1981; paderes *et al.*, 1992 and Bolar *et al.*, 2000).**

Bacillus subtilis appeared in the first rank before *Trichoderma* sp. This might be due to that *B. subtilis* act through production of number of antibiotic. **Ferreira *et al.*, (1991); Aska, and Shoda, (1996) and Farahat, (1998)** who stated that this bacteria produce bacterocin and subtilisin antibiotics

In these studies, all bio control agents and other reatments were screened to compare their effect on powdery mildew disease severity. Data obtained showed that, *Bacillus* subtilis and *T. harzianum* were the most effective treatments. This can be explain in the light of research work carried by **Abd El-Moity (1981),**Who stated that some isolates of *T. harzianum* work through different mechanism i.e. production of Gliotoxin, mycoparasitism and grow very fast and act as barrier between susceptible plant tissues and virulent pathogens. *T. harzianum* in addition to *Bacillus subtilis,* chicken manureand garlic extract gave considerable control. Highly significant differences were recorded when any of these treatments was compare with control treatment

In general and through data obtained from these studies it is clear that .The most effective factor to control powdery mildew in marigold was when *Bacillus subtilis* was used only 6.38% disease severity was obtained. *Trichoderma harzianum* occupied the second rank and only 10.07 % disease severity was obtained. Using chicken manure suspension came in the third rank cause reduction in disease severity compare with control treatment and only 14.53% disease severity was recorded .Topaz occupied the fourth rank and 17% disease severity was obtained . Garlic came after topaz 17.82 % disease severity was recorded. Using Mixture of Micronutrient plus Potassium and Calcium has less effect and occupied the lost rank and 23.13 % disease severity was recorded

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المقاومة البيولوجية لمرض البياض الدقيقي في نبات الأقحوان في محافظة الفيوم

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- يعتبر الأقحوان من أهم النباتات التصديرية في مصر وقد بلغت المساحة المزروعة عام ٢٠٠٥ حوالي ٥٥٠ فدان أنتجت ٩٣٩٧ طن من الأزهار.
- يصاب الأقحوان بالعديد من الأمراض الهامة في مراحل نموه من بينها مرض البياض الدقيقي.
- استهدفت هذه الدراسة استيضاح دور المقاومة غير الكيماوية والكيماوية في مقاومة مرض البياض الدقيقى على الأقحوان وقد تمت هذه الدراسة تحت ظروف المعمل والحقل.

ويمكن تلخيص أهم النتائج المتحصل عليها في النقاط التالية :

١- تم الحصول على كائنات مختلفة من جراء العزل من منطقة الPhylopllane في نباتات الأقحوان السليمة والتي تم تعريفها في معهد بحوث أمراض النبات بمركز البحوث الزراعية وهذه العزلات استخدمت كمضادات في هذه الدراسة.

٢- اختلفت العزلات المختبرة في تأثيرها ضد المسبب المرضي وعموما وجد أن فطر التريكودرما هارزيانم وبكتيريا باسلس ستلس كانت أكثر المضادات فاعلية ضد المسبب المرضي.

٣- أظهرت النتائج أن نسبة حدوث المرض وأيضا شدته قد انخفضتا بالرش بالتريكودرما هارزيانم وباسلس ستلس بالإضافة إلى ذلك حدثت زيادة في متوسط عدد الأزهار ومتوسط وزن الأزهار للنبات الواحد.

٤- أظهرت النتائج أن أفضل وقت لاستخدام عوامل المقاومة الحيوية كان كل أسبوع.

٥- أظهرت النتائج أن أفضل تركيز من عوامل المقاومة الحيوية كان ٢٠ مل لكل لتر ماء.