Biological Control of Powdery Mildew on Zinnia (*Zinnia elegans*, L) Using Some Biocontrol Agents and Plant Extracts

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Abstract

Powdery mildew is a potentially serious disease of Zinnia (*Zinnia elegans*). The disease is caused by the air-borne fungus *Erysiphe cichoracearum* and it is an occasional disease of Zinnia in Egypt. Culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus* and an actenomycetal isolate in addition to two plant extracts i.e. miswak *(Salvadora persica*) and henna (*Lawsonia inermis*) were evaluated to control powdery mildew disease of Zinnia plants. Culture filtrates of the aforementioned biocontrol agents were used as 25 and 50%. Bioassays were conducted under field conditions during the two successive seasons of 2006 and 2007 at the Experimental Farm of the Fac. of Agric., Kafr El-Sheikh Univ., Egypt to test the efficacy of these culture filtrates applied to protect Zinnia plants from powdery mildew disease.

Spraying zinnia plants four times, beginning from June 15th with one week interval by a hand atomizer under field conditions with the previously culture filtrates and the two plant extracts gave sufficient control to powdery mildew disease. The obtained results showed that, all used treatments led to significant decrease in both disease incidence and severity compared with control treatment. The highest disease incidence inhibition was obtained when *Epicoccum* sp. and *T. harzianum* were used as 50% (v/v) sterilized water followed by henna and miswak extracts were used, respectively. Sprayed plants recorded best results for most growth characters, peroxidase (POX) and polyphenol oxidase (PPO) enzymes activity compared unsprayed one. In conclusion, biocontrol agents and some plant extracts can be substitutes to fungicides as an alternative and safe method for controlling powdery mildew disease of Zinnia.

Keywords: *Zinnia elegans,* Powdery mildew, Biological control, Biocontrol agents

1. Introduction

Zinnia *(Zinnia elegans*) is an herbaceous summer annual flower blooming from mid-summer all the way until frost and belongs to Family Asteraceae. Zinnias are true American natives that originated from the Southwest US, Mexico and Central America. Hybridizers have turned it into one of the most popular cut flower or bedding plants. However, Zinnia plants are attacked with various diseases causing losses in cut flowers yield, Powdery mildew caused by *Erysiphe cichoracearum* is one of the most serious diseases of Zinnia in Egypt.

Application of chemical fungicides to protect plants from the attack of the pathogenic fungi was the primary means for controlling diseases. In recent years chemical fungicides have become less effective due to the development of pathogen resistance beside the potentially undesirable effects of the fungicides on human, plants and other beneficial organisms (Thomas, 1986 and Manandhar *et al*., 1988). Biological control methods could be considered as an alternative of chemical control. Successful biological control of foliar diseases has been achieved by a number of researchers under greenhouses and field trials using fungal and bacterial antagonists (Singh *et al*., 2000; Abd El-Moneim, 2001; Mosa, 2002; El-Gamal, 2003; Kamel, 2003,;Saber *et al*., 2003; Mc Grath, 2004 and Hussein *et al*., 2007). Plant extracts were recently used to control many pathogens and diseases (El-Shoraky, 1998 and El-Kazzaz *et al*., 2003). Plant extracts and biocontrol agents induced host resistance through increased activity of many enzymes such as peroxidase and poly phenol oxidase which playing a

defense role against invading pathogens (Kohl and Fokkema, 1998; Caruso *et al*., 2001 and Nawar and Kuti, 2003).

The current investigation was planned to study the ability of cell free culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus* and an actenomycetal isolate in addition to two plant extracts, extracted from miswak (*Salvadora persica*) and henna (*Lawsonia inermis*) to control powdery mildew disease of Zinnia plants under field conditions.

2. Material and Methods

Seeds were sown in nursery beds on March 15th in both seasons and seedlings were transplanted in May 1st to a clay soil in plots 1x 1.5m^2 at 50 cm apart as twins in the hill, and each bed was divided into two parts (1x0.75 m^2), so each part contained 12 plants (6 hills) and considered a replicate. Therefore, every treatment consisted of 36 plants (18 hills) in the three replicates. The experiment was arranged in a completely randomized block design. Plants including control were fertilized with N, P and K at rates of 100, 200 and 100 kg/ fed., respectively beginning from May 15th and repeated three times with two weeks interval. The used fertilizers were ammonium sulphate "20% N", calcium super phosphate "15.5% P_2O_5" and potassium sulphate "48% K_2O". The common agricultural practices i.e. watering, weeding control, etc. were done whenever plants needed.

2.1 Culture filtrates

Culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus* and an actenomycetal isolate were obtained by growing these isolates on PD broth for 15 days at 26-28 ˚C, and then centrifuged at 5,000 rpm for 15 min., then the supernatant was filtered by filter glass (El- Boghdady, 1993). After that filtrates were diluted at 25 and 50% (v/v) by a sterilized water. These aforementioned isolates were previously isolated and identified by El-Kot (2007).

2.2 Preparation aqueous extract of henna and miswak

Leaf samples (100 gm) of henna (*lawsonia inermis*) powdered was macerated with 100 ml distilled water for 24 h at 4°C. The macerate was centrifuged at 4000 rpm for 30 min, then filtered through filter paper and served as the mother extract. The mother extract was diluted to 25% with distilled water and sprayed on the plant (Satish *et al*., 2007).

Twenty grams of miswak (*Salvadora persica*) stem powdered were soaked in 100 ml of distilled water for 24 h at 4°C. The mixtures were centrifuged at 2000 rpm for 10 min., and the extract was filtered through filter paper and sprayed on the plant (Al Bagieh *et al*. 1994).

2.3 Control of powdery mildew under field conditions

Under field conditions culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus* and an actenomycetal isolate (25 and 50% v/v sterilized water) in addition to two plant extracts of *Salvadora persica* and *Lawsona inerms* were used to control powdery mildew disease of Zinnia plants.

The prepared microbial culture filtrates and plant extracts were introduced to the experimented plants. Culture filtrates and plant extracts were amended with calculated aliquots of an adhesive surfactant (New-Film 1265 registered by Ministry of Agric. and Reclamation Lands, Egypt) as recommended (30 ml/100l) and hand homogenized before fine spraying on the experimental plants (Abd El-Moneim, 2001). Plants were sprayed with each of aforementioned treatments as soon as the first signs of the symptoms were observed. For control treatments, plants were sprayed with water and in the other treatments, Kimazien 75% as a fungicide was also used. Percentage of disease incidence and severity were determined after 7 days from the last spray according to the scale reported by Horsfall and Barrett (1945) and Biswas *et al.,* (1992).

2.4 Enzymes extraction and assay

Leaves samples from each zinnia plants treatment, healthy and infected were collected 24h after sprayed with culture filtrates of *T. harzianum, Epicoccum* sp, *Str. Endus*, one isolate of actenomycetes, plant extracts of *S. persica* and *L. inerms* for peroxidase and polyphenol oxidase enzymes activity assay. In addition, untreated healthy and infected leaves were used as control. Enzyme extract was obtained by grinding leaf tissue in 0.1 M sodium phosphate buffer at pH 7.1 (2m/g leaf tissues) in a porcelain mortar. The extracted tissues were strained through four layers of cheesecloth. Filtrates were centrifuged at 3000 rpm for 20 min. at 6°C. The clear supernatants were collected and considered as crude enzyme extract. Peroxidase (POX) activity was determined according to the method of Allam and Hollis (1972) by measuring the oxidation of pyrogallol to pyrogalline in the presence of hydrogen peroxide. Peroxidase activity was measured following the changes in absorbance at 425 nm every 1 min. up to 4 minutes. Polyphenol oxidase (PPO) was determined according to Maxwell and

Batman (1976). The changes in absorbance was following spectrophotometrically measured at 495 nm, and recorded every 1 min. up to 4 min. All measurements were assayed using Beckman Spectrophotometer Du®7400.

At the end of the experiment, the following data were recorded (average of both seasons):

1. Growth parameters as plant height (cm), branch number/ plant, leaf area (cm2), shoots fresh and dry weights /plant (g), root length (cm) and roots fresh and dry weights /plant (g), flower number /plant, flower diameter (cm) and flower fresh and dry weights (g).

2. Total green colour (SAPD) was measured using a portable chlorophyll meter (Minolta SPAD- 502, Japan).

Means between treatments were compared with Duncan's Multiple Range Test according to Snedecor and Cochran **(1982).**

3. Results

3.1 Effect on disease incidence and severity

Data presented in Table (1) showed that all used treatments were significantly decreased disease incidence (average number of powdery mildew spots/leaf) and disease severity (percent of surface infected area) on Zinnia plants. Results indicated that, the best control of the studied disease was obtained when culture filtrates were sprayed on Zinnia plants as 50% (v/v) sterilized water. Meantime, culture filtrates of *Epicoccum* sp. as 50% was the most efficient treatment on disease incidence and severity in the two successive seasons. It significantly decreased disease and severity from 30.78-2.03%, 87.2-6.4%, respectively in 2006 season and from 35.69-2.04%, 91.5-6.8%, respectively in 2007 season followed by cell free culture filtrates of *Trichoderma harzianum*, since it decreased disease incidence and severity from 30.78-2.08%, 87.2-7.7%, respectively in 2006 season and from 35.69-3.00%, 91.5-8.1% respectively in 2007 season. However, culture filtrates of *Streptomyces endus* was the least effective treatment used as either 25% or 50%. In general, all used treatments gave better or similar results with those obtained when the fungicide Kimazien 75% used.

3.2 Effect on peroxidase and polyphenol oxidase activity

The effect of spraying zinnia plants with culture filtrates of *T. harzianum, Epicoccum* sp., *Str. Endus*, one isolate of actenomycetes, *S. persica* and *L. inerms* extracts on peroxidase and poly phenol oxidase enzymes activity was determined after 24 h from last spraying. In this study, results showed that peroxidase and polyphenol oxidase activities were significantly increased as a result of spraying Zinnia plants with these treatments (Tables, 2 and 3).

The highest activity of peroxidase was observed when culture filtrates (50%) of *Epicoccum* sp. was sprayed on zinnia plants (1.747, 1.756, 1.768, 1.777) followed by plant extract of *L. inerms* (1.734, 1.741, 1.746, 1.758) compared with control (0.403, 0.405, 0.409, 0.413). Similarly, culture filtrates (50%) of *Epicoccum* sp. caused the highest activity of polyphenol oxidase than other treatments as gave 0.186, 0.220, 0.224 and 0.227 followed by *T. harzianum* at 50% as gave 0.115, 0.130, 0.0134 and 0.140 against 0.043, 0.071, 0.073 and 0.077 for control (Table, 3). Meantime, results indicated that, the high concentration of used culture filtrates caused higher activity of both peroxidase and polyphenol oxidase than the lowest one. For example peroxidase activity caused by spraying Zinnia plants with 50% culture filtrates of *T. harzianum* was 1.007, 1.020, 1.037, 1.059 compared with 0.501, 0.506, 0.511, 0.517 which was caused by spraying with 25%.

3.3 Effect on some growth and flowers aspects

3.3.1 Plant height, branches number and leaf area

The significantly tallest plants resulted from the treatment of *Epicoccum* sp. at both 50 and 25% as recorded 155.19 and 149.74cm, respectively (Fig. 1) followed by *Streptomyces endus* at 50% and Kemah zein 75% as gave 143.12 and 139.70cm, respectively. The shortest plants were that treated with *Trichoderma harzianum* at both percentages 25 and 50% and distilled water (control) as gave 116.18, 118.62 and 90.88 cm, respectively.

The highest number of branches resulted from the plants treated with *Epicoccum* sp. at 50% and Kemah zein 75% as recorded 9.69 and 8.06, respectively. The least branch number resulted from the plants treated with *Lawsonia inerms* and *Salvadora persica* extracts as recoded 5.58 and 5.54, respectively against 3.86 for control (distilled water). As for leaf area, it is obvious that the corresponding treatment at both 50 and 25% gave the widest leaves compared to the other treatments as recorded 39.55 and 37.13cm^2 followed by the treatments of isolate of actinomycete at 50% and *Lawsonia inermis* extract as recorded 36.68 and 36.39 cm^2, respectively. The narrowest leaves resulted from plants treated with *Salvadora persica* extract and distilled water (control) as recorded 31.29 and 24.41cm^2, respectively.

3.3.2 Fresh and dry weights of shoots and root length

It is evident from Fig.2 that plants treated with *Epicoccum* sp. at both 50 and 25% followed by isolate of actinomycete at 50% and Kemah zein 75% gave the heaviest fresh shoots as recorded 308.59, 303.36. 301.64 and 301.62g/plant, respectively. Whereas, the heaviest shoots dry weight resulted from plants treated with isolate of actinomycete at both 50 and 25%**,** *Streptomyces endus* at 50% as gave 40.09, 39.18 and 39.35g/ plant, respectively. The lightest fresh and dry shoots resulted from plants treated with *Salvadora persica* extract and distilled water (control) as recorded 177.60 and 105.89g fresh weight/plant and 19.68 and 10.24g dry weight/ plant. The other treatments gave a gradually intermediate values without significant differences among them in most cases.

As for root length, it is clear that plants treated with *Epicoccum* sp. at both 50 and 25% followed by isolate of actinomycete at 50% gave the tallest roots as recorded 16.21, 14.78 and 14.39cm, respectively against 8.60cm for control (distilled water).

3.3.3 Fresh and dry weights of roots

All treated plants gave a significantly heavier fresh and dry roots than control (distilled water) (Fig. 3). The treatment of *Epicoccum* sp. at 50% gave the highest values for both roots fresh and dry weights as recorded 13.74 and 1.94g /plant, respectively. This was followed by plants treated with isolate of actinomycete at 50% and Kemah zein 75% for roots fresh weight as recorded 13.18 and 13.16g/ plant, and that treated with *Streptomyces endus* at 50% and Kemah zein 75% for roots dry weight which recorded 1.85 and 1.84g/ plant, respectively. The lowest values obtained from plants treated with *Salvadora persica* extract and distilled water (control) for roots fresh weight and *Lawsonia inermis* extract and distilled water (control) for roots dry weight as recorded 12.07, 9.98, 1.69 and 1.37g/ plant, respectively

3.3.4 Flower number

It is clear from Fig. 3 that the highest number of flowers obtained when plants treated with *Epicoccum* sp. at both 50 and 25% followed by Kemah zein 75% and isolate of actinomycete at 50% as gave 23.40, 21.87 and 21.57, respectively. The least flower number observed on plants treated with *Lawsonia inermis* extract or distilled water (control) which recorded 14.87 and 12.13, respectively.

3.3.5 Flower diameter and flower fresh and dry weights

The biggest flowers were observed on either plants treated with isolate of actinomycete or *Epicoccum* sp. at both 50 and 25% without significant differences among them as gave 6.94, 6.68, 6.76 and 6.77cm, respectively (Fig, 4). The smallest flowers (4.73 and 3.65cm) resulted from plants treated with *Salvadora persica* extract and control (distilled water).

As for flower fresh and dry weights Fig. 4 refer that the heaviest flower fresh and dry weights resulted from plants treated with either *Epicoccum* sp. or isolate of actinomycete at 50% as recorded 4.87 and 4.59g fresh weight and 0.97 and 0.98g dry weight, respectively. The lightest flower fresh weight (2.92 and 2.06g) resulted from plants treated with *Salvadora persica* extract and control (distilled water) whereas, plants treated with *Trichoderma harzianum* at 25% and distilled water (control) gave the lightest flower fresh weight (0.51 and 0.42g).

3.4 Effect on total green colour

The greenest plants were that treated with either *Epicoccum* sp. and *Streptomyces endus* each at 50% or Kemah zein 75% without significant differences as recorded 34.61, 34.53 and 34.24SAPD, respectively. The pale leaves were noticed on plants treated with *Trichoderma harzianum* at 25% and distilled water (control) as recorded 29.67 and 26.39SAPD, respectively.

4. Discussion

The present work was designed to reduce using chemicals in agriculture process and find out the most suitable non-chemical method to protect Zinnia plants against powdery mildew disease. In this study, data obtained showed that all used culture filtrates significantly reduced disease incidence and severity of zinnia powdery mildew accompanied with improving the most studied growth characters and total green colour. These results might be due to that *Epicoccum* sp. and *Trichoderma harzianum* inhibit disease by producing some antifungal substances, i.e. gliotoxin and some growth regulators. These compounds are amphilic, membrane active surfactants and when sprayed on plant surface, prior infection led to stimulate plant resistant and enforce treated plants to produce some metabolites which depress the pathogen and some growth promoters such as indols which increased plant growth generally (Abd El-Moity, 1981; Abd El-Moity, 1985; Sanker and Jeyarajan 1996;

Reguchander *et al*., 1997; Elad *et al*., 1998; Umseha *et al*., 1999; Bolar *et al.,* 2000; Howell *et al.,* 2000; El-Gamal, 2003; Saber *et al*., 2003; El-Kot, 2007 and Hussein *et al*., 2007).

The reduction in disease incidence and severity of Zinnia powdery mildew with spraying *Salvadora persica* and *Lawsona inermis* extracts might be due to the antimicrobial activities towards the studied pathogen. These results are consistent with previous investigations (Farag *et al.*, 1989; Mc Cutheon *et al*., 1994; Navarro *et al.*, 1996; El-Kazzaz *et al*., 2003 and Satish *et al*., 2007). Culture filtrates and plant extracts gave an increment of peroxidase and polyphenol oxidase enzymes activity which indicate a positive relationship between increases in peroxidase and polyphenol oxidase enzymes activity and reduction in disease incidence and severity of Zinnia powdery mildew.

Many investigators supported this idea since they stated that there are positive relationships between peroxidase enzyme and resistance developed in plants (Deacon and Berry, 1993 and Kohl and Fokkema, 1998, Nawar and Kuti, 2003 and Emeran *et al.,* 2006). Similarly, Caruso *et al*., 2001 stated that peroxidase enzyme is playing a defense role against invading pathogens of wheat kernels. Other investigators reported that peroxidase is known to be involved in the oxidation of polymerization of hydroxycinnamyl alcohols to yield lignin and cross-linking isodityrosine bridges in cell wall, peroxidase also produces free radicals and hydrogen peroxide which are toxic to many microorganisms (Vance *et al*., 1980; Fry, 1982 and Pena and Kuc, 1992). Also, Ride,1983 and Tarrad,1993 stated that increase in peroxidase activity enhances lignification in response to infection with pathogens which may restrict fungal penetration.

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Table 1. Effect of culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus*, one isolate of actenomycetes (25 and 50% v/v sterilized water) and plant extracts of *Salvadora persica* and *Lawsona inermis* on disease incidence and severity of powdery mildew of zinnia plants

Treatments	Application rats	Average no. of spots/leaf (Diseaseincidence)		Percent of surface infected area (Disease severity)	
		2006	2007	2006	2007
Trichoderma harzianum	25%	3.61i	4.02i	12.6g	13.2g
	50%	2.08k	3.00k	7.7k	8.1j
Streptomyces endus	25%	6.10b	7.06b	16.5c	17.6c
	50%	5.31d	5.20f	14.8d	13.9f
Epicoccum sp	25%	4.20h	3.11j	9.6h	8.7i
	50%	2.03l	2.04l	6.4l	6.8l
Isolate of actinomycete	25%	5.12e	6.35c	19.3b	20.1b
	50%	3.21j	5.02g	14.5e	16.2d
Miswak extract	-	5.54c	6.25d	8.9i	10.2h
Henna extract	-	4.49g	4.26h	7.9j	7.2k
Kemah zein 75%	2g/l	5.03f	6.11e	12.9f	14.2e
Distilled water (control)	-	30.78a	35.69a	87.2a	91.5a

Means within a column having the same letters are not significantly different according to Duncan,s Multiple Range Test.

Table 2. Activity of peroxidase in leaves of Zinnia plants after 24 hrs from treating with culture filtrates of *T. harzianum, Epicoccum* sp., *Str. Endus*, one isolate of actenomycetes and plant extracts of *S. persica* and *L. inermis*

Treatments	Application rats	Peroxidase activity/minute			
		1	2	3	4
Trichoderma harzianum	25%	0.501g	0.506f	0.511g	0.517g
	50%	0.007l	0.020j	0.037k	0.059k
Streptomyces endus	25%	0.811d	0.816b	0.827b	0.835b
	50%	0.625b	0.635e	0.642f	0.644f
Epicoccum sp	25%	0.921c	0.928a	0.933a	0.941a
	50%	0.747a	0.756c	0.768c	0.777c
Isolate of actinomycete	25%	0.655f	0.659d	0.664e	0.670e
	50%	0.345j	0.449g	0.466h	0.476h
Miswak extract	-	0.434h	0.460g	0.469h	0.483h
Henna extract	-	0.734e	0.741c	0.746d	0.758d
Kemah zein 75%	2g/l	0.112k	0.115i	0.121j	0.125j
Distilled water (control)	-	0.403i	0.405h	0.409i	0.413i

Means within a column having the same letters are not significantly different according to Duncan,s Multiple Range Test.

Table 3. Activity of polyphenol oxidase in leaves of Zinnia plants after 24 hrs from treating with culture filtrates of *T. harzianum, Epicoccum* sp., *Str. Endus*, one isolate of actenomycetes and plant extracts of *S. persica* and *L. inermis*

Treatments	Application rats	Peroxidase activity/minute			
		1	2	3	4
Trichoderma harzianum	25%	0.080de	0.084d	0.085e	0.088ef
	50%	0.115b	0.130b	0.0134b	0.140b
Streptomyces endus	25%	0.078de	0.085d	0.089de	0.094def
	50%	0.113b	0.126b	0.130b	0.133g
Epicoccum sp	25%	0.102bc	0.105c	0.111c	0.115c
	50%	0.186a	0.220a	0.224a	0.227a
Isolate of actinomycete	25%	0.069e	0.075d	0.079e	0.084f
	50%	0.118b	0.119bc	0.121bc	0.122c
Miswak extract	-	0.084de	0.102c	0.103cd	0.105cde
Henna extract	-	0.094cd	0.104c	0.105cd	0.107cd
Kemah zein 75%	2g/l	0.082de	0.113bc	0.117bc	0.119c
Distilled water (control)	-	0.043f	0.071d	0.073e	0.077f

Means within a column having the same letters are not significantly different according to Duncan,s Multiple Range Test.

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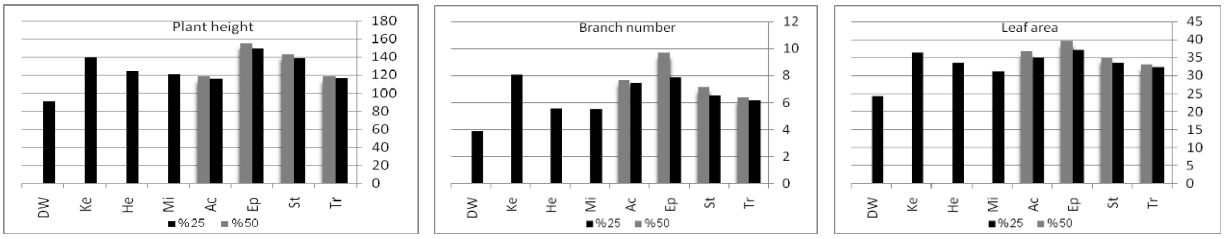
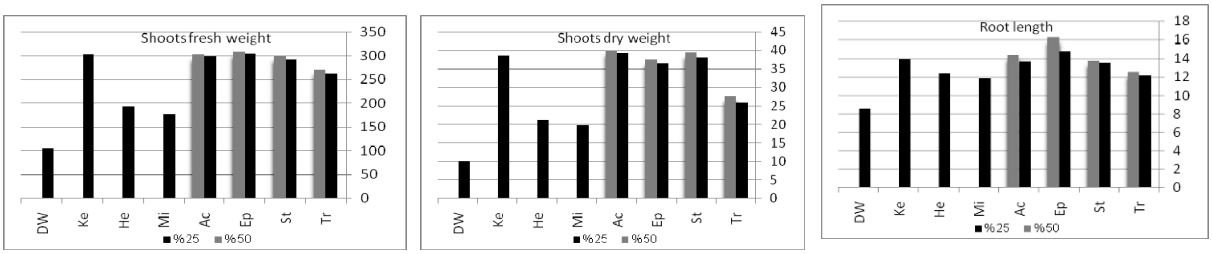


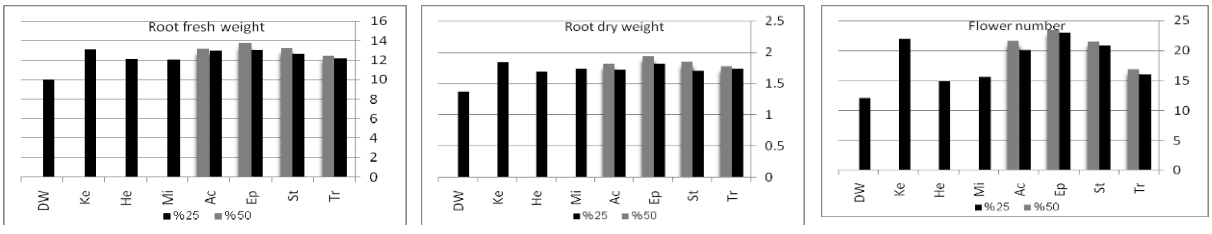
Tr: *Trichoderma harzianum, St: Streptomyces endus, Ep: Epicoccum* sp, *Ac:* isolate of actenomycetes, Mi:Miswak (*Salvadora persica),*He: Henna (*Lawsona inermis*), Ke: Kemah zein 75% and DW: Distilled water (control).

Figure 1. Effect of culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus*, one isolate of actenomycetes (25 and 50% v/v sterilized water), plant extracts of *Salvadora persica* and *Lawsona inermis* and Kemah zein 75% on plant height (cm), Branch number/ plant and leaf area (cm^2) of *Zinnia elegans*, L. (average of both seasons)



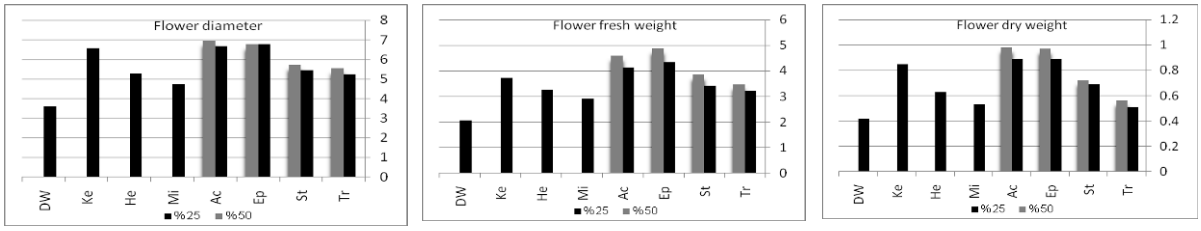
Tr: *Trichoderma harzianum, St: Streptomyces endus, Ep: Epicoccum* sp, *Ac:* isolate of actenomycetes, Mi:Miswak (*Salvadora persica),*He: Henna (*Lawsona inermis*), Ke: Kemah zein 75% and DW: Distilled water (control).

Figure 2. Effect of culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus*, one isolate of actenomycetes (25 and 50% v/v sterilized water), plant extracts of *Salvadora persica* and *Lawsona inermis* and Kemah zein 75% on shoots fresh and dry weights (g)/plant and root length (cm) of *Zinnia elegans*, L. (average of both seasons)



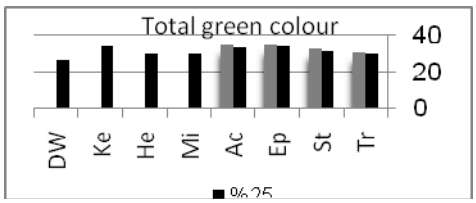
Tr: *Trichoderma harzianum, St: Streptomyces endus, Ep: Epicoccum* sp, *Ac:* isolate of actenomycetes, Mi:Miswak (*Salvadora persica),*He: Henna (*Lawsona inermis*), Ke: Kemah zein 75% and DW: Distilled water (control).

Figure 3. Effect of culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus*, one isolate of actenomycetes (25 and 50% v/v sterilized water), plant extracts of *Salvadora persica* and *Lawsona inermis* and Kemah zein 75% on roots fresh and dry weights (g)/ plant and flowers number of *Zinnia elegans*, L. (average of both seasons)



Tr: *Trichoderma harzianum, St: Streptomyces endus, Ep: Epicoccum* sp, *Ac:* isolate of actenomycetes, Mi:Miswak (*Salvadora persica),*He: Henna (*Lawsona inermis*), Ke: Kemah zein 75% and DW: Distilled water (control).

Figure 4. Effect of culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus*, one isolate of actenomycetes (25 and 50% v/v sterilized water), plant extracts of *Salvadora persica* and *Lawsona inermis* and Kemah zein 75% on flower diameter (cm) and flower fresh and dry weights (g) of *Zinnia elegans*, L. (average of both seasons)



Tr: *Trichoderma harzianum, St: Streptomyces endus, Ep: Epicoccum* sp, *Ac:* isolate of actenomycetes, Mi:Miswak (*Salvadora persica),*He: Henna (*Lawsona inermis*), Ke: Kemah zein 75% and DW: Distilled water (control).

Figure 4. Effect of culture filtrates of *Trichoderma harzianum, Epicoccum* sp., *Streptomyces endus*, one isolate of actenomycetes (25 and 50% v/v sterilized water), plant extracts of *Salvadora persica* and *Lawsona inermis* and Kemah zein 75% on total green colour (SAPD) of *Zinnia elegans*, L. (average of both seasons).

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