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Integrated management of Rhizoctonia Root rot disease infecting strawberry in Egypt

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ABSTRACT

Root rot disease of strawberry caused by *Rhizoctinia* spp.is a common disease in commercial fields in Egypt.This study was conducted to identify the pathogen. Testing four strawberry cultivars for their susceptibility toRhizoctonia root rot disease and test the efficiency of (bio-control agents, soil mulch, antioxidant, chemical inducer and fungicides) to control the disease. The obtained data showed that all tested antagonistic and mulching soil significantly reduces root rot and increased fruit yield. The severity of infection was reduced when different antioxidant andchemical inducer were applied to the soil compared with the untreated soil. The combination between black mulch and fungicides was the best treatment compared to the other treatments. Integrated control significantly decreased the percentage of infected plants using (Black mulch+ Boscalid+ *Trihoderma hamatum* + Salisylic acid) followed by (Boscalid+ Black mulch) was the most effective treatment in controllingRhizoctonia root rot disease on cv. Sana strawberry plants.

Key words: Soil borne diseases**,** Strawberry, Root rot, *Rhizoctonia solani*, *Rhizoctonia fragariae*,Biological control, Chemical controland integratedcontrol.

INTRODUCTION

Strawberry (*Fragaria* x*ananassa* Duch.) is one of the most important cropsworldwide and one of the majorwinter vegetable crops in Egypt. It is reported that strawberry plants are attacked by several soil borne pathogens causing severe losses such as *Fusarium* spp., *Rhizoctonia* spp. and *Macrophomina phaseolina* (Fahim *et al.,*1994 and El-Sharkawy, 2006), these fungi causing wilt, black root rot,and rot diseases (Fang *et al.,*2011 and Fang *et al.,*2012). Black root rot disease is considered as a complex disease caused by more than one fungus such as *Fusariumo xysporum* (Juber *et al*., 2014), *Macrophomina phaseolina* (Hutton *et al.,* 2013) and *Rhizoctonia fragaria*e (Fang *et al.*, 2013). *Rhizoctonia fragaria* and *Rhizoctonia solani* associated with severe economic losses and serious threat to commercial strawberry plantations, like those have been reported in Japan, Italy and Australia (Matsumoto and Yoshida 2006; Manici and Bonora 2007and Fang *et al.,*2011). Many ways were used to control black root rot. Many fungicides were commonly used in control program such as Rizolex and Tachigaren, they were highly specific against *R. Solani* (Abd-El Kareem *et al.,* 2004; Fayadh *et al.,* 2008; El Morsi and Mahdy, 2013). Also, biological control was used strawberry soil borne diseases in many investigations like Gliocladium and Trichoderma (Vestberg *et al.,*2004) or *Trichoderma* spp. (Porras *et al.,* 2007a,b). Moreover,it was reported that *Trichoderma harzianum* reduced the disease severity of black root rot of strawberry plants (Elad *et al.,*1983). Ciccarese *et al.,* (1985) concluded that *T. viride, T. Hamatum* and *T. Harzianum* showed antagonistic activities against *R. fragaria, F. solani,*the causal agents of strawberry root rot. On the other hand, Sugimura *et al*., (2001), Bletsos *et al*., (2002) and Umang and Harender (2004) reported that using the transparent polyethylene mulch (25µm) white or black for 40 days was effective incontrolling strawberry wilt and root rot diseases.Galal and Abdou (1996) reported

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that application of antioxidants to soil was more effective in controlling fusarium diseases. El-Kolaly (2003) tested the effect of four antioxidants *i.e.,* Ascorbic acid, Citric acid, Mannitol and Salicylic acid against root and crown rot of strawberry. Salicylic acid and Ascorbic acid was the most effective antioxidants on disease development. Mansour (2005) stated that antioxidants were significantly effective in improving disease control and fruit yield production of strawberry than the control. Osman *et al*., (1991) found that tolclofos-methyl at 4 ppm followed by benomyl at 8 ppm were more effective against *R. fragariae in vitro* than dichlofluanid, copper exyquinolate + carboxin or pencycuron. Dipping transplants in 250-ppm benomyl suspension before transplanting effectively reduced the infection of strawberries. Fazli-Raziq and Fox (2006) studied the effects of the sequential application (at 40-day intervals) of fungal antagonists and systemic fungicides (fosetyl-Al [fosetyl] and fenpropidin) on the canopy of strawberrycv. Cambridge Favourite. The antagonists and fungicides did not vary significantly, although the sequences of application significantly differed in terms in an integrated control strategy, the antagonists should be applied after fenpropidin, allowing reasonable time for toxicity of the chemical to diminish.

This studyaimed to avoid the hazards of using fungicides throughfinding an alternative control method such as biological control, soil mulch, induced systemic resistance either individual or in form of integrated control programs

MATERIALS AND METHODS

Source of strawberry root rot pathogens:

Sample collection

Infected strawberry plants showing root rot symptoms were collected from different districts (Queisna, Menouf, Ashmon and El-Bagour) in Menoufiagovernorate during two successive seasons (Table1). Root rot pathogens which inhibitingthe infected strawberry rootswere isolated on PDA medium.The resultantcultures were purified using single spore culture or hyphal tip techniques. The pure cultures of growing fungi were then examined microscopically and identified according to their taxonomic features in Agric. Bot. Dept., Fac. Agric., Menoufia Univ.,as illustrated by Neergard (1945), Barnett (1960) and Domsch *et al.*, (1980).The most frequently root rot fungi were used in pathogenicity test.

Pathogenicity test andVarietal reactions:

Pathogenicity test with different fungal isolates was conducted in order to confirm their virulence to define the most aggressive fungal isolate causing the most serious damage on strawberry plants due to root rot under field conditions.

The most frequent pathogenic fungi were *Rhizoctonia fragariae* and *Rhizoctonia solani* (Table 1) that isolated fromdifferent districts, were used in diseased sample collection fromcasual's isolation.Four isolates of eachfungus (*R. solani* and *R. fragariae*) representing the four districts were tested against four strawberry cultivars (Sana, Fertuna, Florida and Festival) to test the most susceptible cultivar (Table 2).

Source of Trichoderma isolates:

Trichoderma fungi were isolated from soil and rhizosphere samples of grown strawberry in the previously mentioned fields by uprooting the infected plants with great care to obtain most of the intact root system. The dilution plate method (DPM) was used for isolation of *Trichoderma* spp. The isolated *Trichoderma* fungi were cultured onto 20% malt extract agar, incubated for two days at 25°C then, identified according to Rifai, (1969) and Bissett, (1991). Stock cultures of isolated *Trichoderma* spp. were maintained on PDA slants then kept in a refrigerator at 5°C and they repeatedly sub-cultured every 4 weeks on fresh PDA slants.

Control studies

a-Biological agents

Primary tests were carried out *in vitro* before application in the field to through light on the real effect of the biological agents on the most aggressive pathogenic *R. fragaria.* Three isolates of *Trichoderma harzianum, T. viride*and *T. hamatum*were usedfor studying their effect on growth reduction of the tested pathogens *in vitro*.

All field experiments were conducted in randomized complete block design with three replicates for each treatment. The field plot was 4 x 4.2 m^2 ($16.8m^2$ = 1/250 feddan) with five rows. The distance between sowing holes about 25cm. Each plot included about 100 plants/plot (20 plants/row).

Field experiments for biocontrol by *T. harzianum* and *T. hamatum* were carried out during the second season using transplants of cv. Sana in naturally infested soil at Shibin El-komdistrict, Menoufia governorate. The bio control agents were used as a soil treatment for disease controlling and on crop yield under natural conditions.The inocula of bio agents were added in each hill one week before transplanting. Each hill received 50g of colonized *T. Harzianum*or *T. Hamatum* ($5X10^7$) then irrigated and left for inoculum propagationand then transplants were planted. Untreated plots with the antagonistic fungi weretransplanted with untreated transplantsto serve as control. Disease incidence was recordedat 90 days from transplanting as percentage of infection and disease index.

b- Soil mulch

Two types of transparent polyethylene plastic i.e. black and white sheets were used for soil mulching three weeks after strawberry transplanting in field soil. Disease incidence was recordedat 90 days from transplanting as percentage of infection and disease index.

c- Antioxidants and Chemical inducer

Four antioxidants andchemical inducer i.e. salicylic acid, ascorbic acid, citric acid and Calcium Chloride ($Cacl_2$) were used. Studies were done under field conditions for determination the suitable concentrations of the tested antioxidants. Field experiments were carried out during the second season. The field soil was naturally heavily infested with the causal pathogens of root rot pathogens. In these experiments, after preparation the land for sowing, Strawberry transplants Sana cultivar was treated with four antioxidants (Salicylic acid, Ascorbic acid, Citric acid, and Calcium chloride).They were used at concentration of 0.2mµ and applied as soil drench or foliar spray. A check treatment transplants were sown in the previous three parts and treated with water as drench or foliar spray. Disease incidence was recorded at 90 days from transplanting as percentage of infection and disease index. The yield of fruits was also estimated.

c-Fungicides

Strawberry transplants Sana cultivar was treated with different fungicides (50% of the recommended dose of Rizolex-T 3g/L and Boscalid 2g/L) by dipping the roots of these transplants in the fungicide solution separately for 15 min. then sown in the previous three parts. Control treatment was consisted of free fungicides strawberry transplants and sown in uncovered soil. Disease incidence was recorded after 90 days from transplantingas percentage of infection and disease severity index. The yield of fruits was also estimated.

d-Integrated control

Field experiments were conducted during the second season growing season at Shebin El-Komdistricts, Menoufia governorate. The experiments were designed to study the effect of the following treatments: (1) black mulch (2) soil infestation with *T.hamatum* (3) soil treatment with Salicylic acid (4) black mulch + Boscalid (5) black mulch +*T.hamatum* (6) black mulch + Salicylic acid (7) Boscalid+ Salicylic acid (8)*T.hamatum* + Salicylic acid (9) black mulch + Boscalid +*T. hamatum* + Salicylic acid.Complete randomized block design with three replicates for each treatment were used. The field plots were prepared as mentioned before.Disease incidence was recorded as follows. The yield of fruits was also estimated.

Disease assessment

Disease incidence assessment in different experiments was carried out through percentage of infection (infection %) and disease severity index (D.I %).

The percentage of infection (infection %) was calculated according to the following formula:

$$\text{Infection \%} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

disease severity index (D.I %) was determined at 90 days after plantation according to a numerical rating system according to Soliman*et al*., (1988) as follows:
0 = no root discoloration, 1 = very slight root discoloration, 2= slight to moderate root discoloration with restricted slight rot lesion in the crown, 3 = extended darker discoloration of roots with moderate crown rot damage, 4 = severe root rot associated with some foliar acropetalchlorosis.
The disease severity was calculated using the following equation:

$$D.S = \frac{(a \times b)}{N \times K} \times 100$$

Where:a = number of infected plants, b = grade of infection,N = number of total plants, K = maximum grade of infection.

The data obtained were subjected to statistical analysisandprepared as one way analysis of variance of the data using MSTAT statistical software. Mean comparisons were made among treatments with Fishers L.S.D. (0.05 and 0.01).

Statistical analysis procedure:

The data obtained were subjected and statistical analyzed was determined by performing a one way analysis of variance of the data using MSTAT statistical software. Mean comparisons were made among treatments with Fishers L.S.D. (0.05 and 0.01).

RESULTS

Isolation and frequency of the associated fungi

Isolation was made from diseased roots of strawberry plants showing typical symptoms of root rot diseases collected from open fields during two successive seasons. They were collected from the above-mentioned four districts representing different growing areas of strawberry in Menoufia governorate.Isolation was madeonPDA medium yielded a group of fungi, either singly or in combinations. Eight pathogenic fungi were represented in both seasonspurified and identified as *Rhizoctonia fragariae, Rhizoctonia solani Fusarium solani*, *Fusarium oxysporum, Fusarium monoliforme*, *Pythium ultimum*, *Verticillium dahliae*, and *Alternaria* spp.

*Rhizoctonia fragariae*was the most frequently isolated fungus occurred showing (30.1) and (31.3) % duringthe two seasons, respectively. Menouf and El-Bagour districts wereshowed the highest values of their frequency

Table (1): Frequency of isolated fungi from roots of strawberry diseased plants, samples collected from four districts in Menoufia governorate during two successive seasons.

Isolate/District	Season 1						Season 2					
	Queisna	Menouf	Ashmon	El-Bagour	Total	Frequency %	Queisna	Menouf	Ashmon	El-Bagour	Total	Frequency %
Rhizoctonia fragariae	30	33	29	33	125	30.1	29	25	30	24	108	31.3
Rhizoctonia solani	30	28	28	29	115	27.7	31	23	21	21	96	27.8
Fusarium solani	19	14	21	25	79	19.0	12	10	15	14	51	14.8
Fusarium moniliforme	8	9	2	1	20	4.8	2	4	8	9	23	6.7
F. oxysporum	12	17	10	16	55	13.3	13	16	15	10	54	15.7
Pythium ultimum	0	1	1	0	2	0.5	0	0	1	0	1	0.3
Verticillium dahliae	5	2	3	0	10	2.4	2	1	0	0	3	0.9
Alternaria spp.	6	2	0	1	9	2.2	8	1	0	0	9	2.6
Total	110	106	94	105	415		97	80	90	78	345	

Isolation from root rotted strawberry plants yielded a group of fungi (8 pathogenic fungi), either singly or in combinations. The most dominant frequently fungi isolated occurred during two successive seasons were *R. fragaria* and *R. solani*. All the tested fungal isolates were pathogenic to strawberry plants and increased root rot disease as well as reduced the healthy survival plants and yielded typical symptoms of root rot (data not shown) according to the tested pathogen confirming Koch's postulates. One isolate of the most two pathogenic fungi isolates was used for control measurements.

Pathogenicity test and Varietal reactions:

Data presented in Table (2) indicate that *R. Fragariae* and *R.solani* isolates were pathogenic to strawberry plants and increased the percentage of disease severity (D.S %) of root rot diseases. Data also showed that all tested isolates yielded typical symptoms of root rot according to the tested pathogen confirmed Koch's postulates. Datashowed in Table (2) also that one isolate of *R. fragariae*(isolate 1) was the most aggressive root rot pathogens isolates; resulted 100% infection on Sana cultivar (the most susceptible cultivar), followed by isolate (1) of *R. solani* (95.3 % infection) for the same cultivar. The least virulent isolate was *R. Solani* isolate (3), followed byisolate (4) on Florida cultivarwith (85.6 and 86.3%, respectively).Sana cultivar reacted as the most susceptible cultivar in comparing to the other three tested cultivars for all Rhizoctoniaisolates; this result paves the way to use Sana cv. for studying the control of root rotexperiments.

Table (2): Virulence of the most frequently fungal isolates against four strawberry cultivars (Sana, Fertuna, Florida and Festival) under field conditions.

Pathogenic fungi	Isolates	Cultivars / D.S%				Mean
		Sana	Fortuna	Florida	Festival	
Rhizoctonia fragariae	1	100	88.2	85.1	90.7	91
	2	88.4	75.2	77.6	78.1	79.9
	3	85.6	81.2	81.1	79.8	81.9
	4	90.5	85.4	84.6	77.9	84.6
Rhizoctonia solani	1	95.3	78.5	81.2	79.4	83.6
	2	94.1	85.4	84.1	83.4	86.8
	3	88.9	76.5	72.5	76.6	78.7
	4	86.3	75.4	72.6	74.1	77.1
Mean		79.9	70.1	69.3	70.3	

Biological control

Data in **Table (3)** indicate that *T.harzianum* and *T. hamatum* were the most effective bio agents which decreased the growth of *R. fragaria*.*T. hamatum* isolate was the most effective one in reducing the growth of *Rhizoctoina fragaria* comparing with *T. Harzianum* and *T. viride*. On the other and, the highest growth reduction% was recorded with *T. hamatum* with clear inhibition zone and appearance of over growthing on growth of *R. fragaria* followed by *T. harzianum* which exhibited wide inhibition zone comparing with *T. viride*. *T. hamatum* isolate was the only one exhibiting over growthing on growth of *R.*

fragaria without appearance of inhibition zone.

Table (3): Effect of *Trichoderma* spp. on growth of *R. Fragaria* the causal organism of strawberry root rot *in vitro*.

Bioagents	*Rhizoctoina fragaria* Bio-interaction			
	Linear growth (mm)	Growth reduction %	Over growth (mm)	Inhibition zone (mm)
T. hamatum	15	83.3	11	—
T. harzianum	18	80	—	3
T. viride	25	72.22	—	2

TwoTrichoderma isolates i.e. *T. harzianum* and*T. hamatum* were selected to study their effects as biological control agents against *R. fragaria*the causal of root rot under field conditions.Data in Table (4) indicated that biological control agents were decreased infections and controlled diseases incidence in comparing with control (plants treated with the pathogenic fungi only). *T. hamatum*was the most bioagent in controlling diseases incidences; in general; followedby*T. harzianum* with 6.8 and 9.6 of D.S% respectively.

Table (4): Effect of *Trichoderma* spp. on diseases incidence of strawberry root rot caused by *Rhizoctoina fragaria*on (cv. Sana) under field conditions.

Bioagent	*R. fragariae*		
	Infection %	D.S %	Yield g/plant
T. hamatum	11.6	6.8	50.4
T. harzianum	15.2	9.6	24.6
Control	89.9	86.4	33.2
L. S. D. at 5% for	Pathogens (F) = 2.4	Bioagents (B) = 2.9	P x B = 3.1

Soil mulch

Data presented in Table(5) show that mulching soil before transplanting decreased the percentage of infection and disease severity index and increased yield of plants compared to the unmulched treatments. Black mulch followed by white mulch showed the best control and gave the highest yield compared to the control.

Table (5): Effect of mulching soil on diseases incidence of strawberry root rot caused by *Rhizoctoina fragaria*on (cv. Sana) under field conditions.

Mulch treatment	*R. fragariae*		
	Infection %	D.S %	Yield g/plant
Black	12.01	7.55	43.02
White	13.4	7.45	44
Without	88.2	82.7	35.02
LSD at 5%	0.74	0.61	0.26

Effect of some antioxidantsand Chemical inducer:

Data presented in Table (6) show that percentage of infection was reduced as a result of treatment by different antioxidants and chemical inducer when applied as soil drench or foliar spray compared to untreated check plants. Salicylic acid was the most effective antioxidant in decreasing the infection. On the contrary, Citric acid was the least effective treatments.

Table (6): Effect of antioxidants and Chemical inducer on root rot of strawberry cv.

Treatments	Antioxidant and Chemical inducer	*R. fragariae*		
		Infection %	D.S %	Yield gm/plant
Soil drench	Salicylic acid	8.55	4.02	47.74
	Ascorbic acid	10.22	5.99	44.02
	Citric acid	8.89	6.33	43.4
	$Cacl_2$	9.50	5.85	40.92
Foliar spray	Salicylic acid	6.50	5.5	38.94
	Ascorbic acid	11.03	8.03	34.22
	Citric acid	10.50	8.66	35.4
	$Cacl_2$	12.33	7.99	33.04
Control		78.9	77.4	23.65
L S D at 5% for:				
Antioxidants (Anti)		1.35	1.4	1.34
Application (Appl)		2.97	2.67	2.17
Anti x Appl		3.26	3.03	2.83

Effect of some fungicides:

The obtained results illustrated in Table (7) show that both tested fungicides as well as their combination were significantly effective in reducing root rot disease infection and disease index also it was increased yield production. The improvement in disease control and yield production was more obvious and significantly better when transplants treated with mixture of fungicides

Table (7): Effect ofsome fungicides on root rot of strawberry cv. Sana grown in naturally infected soil – under field conditions.

Mulch treatments	*R. fragariae*		
	Infection %	D.S %	Yield gm/plant
Boscalid	0.55	0.55	61.24
Rizolex-T	0.78	0.45	60.55
B+R	0.66	1.99	61.89
Control	89.4	88.5	25.66
LSD at 5 %	0.61	0.53	1.62

Integrated control:

Data in Tables (8) demonstrated that all tested treatments for integrated control significantly decreased the percentage of infected plants and increased crop yield compared with control. Black mulch + Boscalid + *T. hamatum* + Salicylic acid followed by Boscalid + Salicylic acid, Black mulch + Boscalid and Boscalid were the most effective treatments in controlling root rot disease on Sana strawberry plants and increased crop yield compared with other treatments and control.Complex of black mulch + Boscalid + *T. hamatum* and salicylic acid was the best treatment for control both diseases and improving total and quality yield.Significant differences were noticed between treatments and disease parameters as well as obtained yield.

Data also showed that, the same integrated treatments, well affected soil borne pathogens, improved growth of strawberry plants and minimized fruit yield loss both in delta and/or reclaimed soil

Table (8): Integrated controls of root rot disease of strawberry cv. Sana under field conditions in 2014/15 season in clay loamy soil at Shibin El-Kom.

Treatments	*R. fragariae*		
	infection %	D.S %	Yield gm/plant
Black mulch	11.89	5.98	44.5
Boscalid	0.62	2.01	65.4
Trichoderma hamatum	11.9	6.2	55.3
Salicylic acid	8.55	2.4	60.4
Black mulch + Boscalid	1.22	0.2	64.3
Black mulch + *T. hamatum*	5.32	0.3	51.3
Black mulch + Salicylic acid	3.58	3.01	62.5
Boscalid + Salicylic acid	0.6	0.5	61.2
T. hamatum +Salicylic acid	2.2	0.56	66.4
Black mulch + Boscalid +*T. hamatum* +Salicylic acid	0.34	0.21	69.4
Control	73.4	78.02	22.5
LSD at 5% for	3.68	3.03	3.94

DISCUSSION

During the two surveying seasons, root rot disease incidence were varied between all locations,these results may be due to the soil type in the different districts as well as the climate conditions (Minufiya governorate agricultural soil included both clay loamy and sandy soil and has a wide range of climate conditions). *Rhizoctonia* species were dominant among 30 genera obtained from roots of strawberry plants collected from natural soil (Watanabe and Inoue, 1980). Other results obtained previously showed that the agricultural soil in different localities in Egypt greatly invaded with *Fusarium* and *Rhizoctonia* spp. both in delta and in reclaimed soils (Awad, 2004).

During the two experiments, mulching soil before transplanting decreased the percentage of infection and increased yield of plants compared with the unmulched treatments. Black mulch followed by white mulch was the best control and gave the highest yield compared with unmulched treatment; it may be due to the variations in temperatures degrees. In general, the combination between black mulch with fungicides was the best treatment for reducing root rotinfection and disease index, also it was increased yield production compared with other treatments. This was true for both types of soil (unpublished results).

The application of antioxidant, Salicylic acid in general, was the best treatment for reducing root rot disease incidence as well as severity and increasing yield productivity compared with the other treatments. Also applications of antioxidants as soil drench was effective in controlling root rot. In this respect, application of antioxidants to the soil was more effective in controlling wilt diseases of some crops than seed treatments Galal and Abdou (1996); Podile and Laxmi (1998); Prachi and Singh (2002); El-Kolaly (2003); Mansour (2005) and El-Sharkawy (2006).

Two experiments of the integrated control concluded that the highly effect of black mulching and bio agents in controlling the disease effects as well as increasing total and quality of strawberry yield may be due to the high temperature available under plastic sheets in winter conditions and the strength of plant growth that favored plants to resist the pathogens in soil. Similar results were obtained by Laugale and Morocko (2000); Tamietti *et al.,* (2000); Sugimura *et al.,* (2001); Forleo (2002); Bletsos *et al*. (2002) and Umang and Harender (2004).

Combination of Black mulch, Boscalid,*T. hamatum*and salicylic acid followed by Boscalid, salicylic acid or Black mulch and Boscalid were the most effective treatments in controlling root rot disease on Sana strawberry plants and increased crop yield compared with other treatments and control. Previous study in Egypt showed that dipping of strawberry cultivars Chandler and Sweet Charlie plants in fungicide solution before planting caused great decrease in percentage of infected plants increased survived plants. Rizolex-T and Vitavax-T completely prevented the infection caused by *Macrophomena phaseolina* and *R. solani* and improved plant growth parameterand yield production. These improvements were more significantly better when transplants treated with fungicides combined to black mulch (El-Sharkawy, 2006).

All tested treatments in integrated control treatments significantly decreased the percentage of infected plants and increased crop yield compared with check. Black mulch + Boscalid + *T. hamatum* + Salicylic acid followed by Boscalid + salicylic acid, black mulch + Boscalid were the most effective treatments in controlling root diseases incidenceof strawberry cv. Sanaand increased crop yield compared with other treatments. Several attempts for integrated control of strawberry root rot and wilt were applied by many investigators (Khosla and Kumar, 2005; Harander and Sharma, 2005 and Fazli-Raziq and Fox, 2006).

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