Effect of different doses of fungicide Bentazole Combl 50% SL on the growth of fungus Nurses *Rhizactonia solani* and *Fusarium solani* isolated from cucumber plant

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Abstract

The study aimed to test the effect of repeated treatment with a different dose of the fungicide Bentazole Combl 50% SL on the appearance of pesticides resistance in pathogenic fungi Rhizactonia solani and Fusarium solani. Pthogenicity tests showed that both Rhizactonia solani and Fusarium solani are pathogenic on radish seeds with infection rates of 100 and 90%, respectively. In case of R.solani, Bentazole Combl 50% SL affected the fungal radial growth, where the lowest radial growth in the treatment of double dose of Bentazole Combl 50% SL compared to the control (6.72 and 0.00) cm respectively, for the first transfer. While, the lowest radial growth appeared in the treatment of the full dose Bentazole Combl 50% SL compared to the control treatment reaching 1.03 cm and 8.15 cm, respectively, for the second transfer.
As for F.solani, the highest radial growth (2.60 cm) occurred in the control treatment and the lowest radial growth (0.00 cm) occurred in the treatment of double dose of Bentazole Combl 50% SL for the first transfer, while in the second transfer, the highest radial growth occurred in the control treatment (4.46 cm) and the lowest radial growth occurred in the treatment of double dose Bentazole Combl 50% SL (0.6 cm).
Similar results were obtained where both fungi grown on the liquid medium (PDB) based on the fresh and dry weight of fungal biomass. Generally, both fungi R. solani and F. solani showed an ability to tolerate and resist the tested fungicide especially when used at lower concentration than the recommended dose.

Introduction

Pesticides have contributed effectively to the reduction of plant diseases and preventing diseases transmition. Studies have shown that it is difficult to obtain economic production without using pesticides in the production process and that the non-use of pesticides leads to a decrease in the production of the agricultural crop by 50-70% .These fungal pathogens vary in their effects on yield quantity and quality depending on environmental conditions such as temperature, humidity and agricultural processes. Fungi are considered the largest and most important group in plant pathology. There are about 8000 species of fungus possessing the ability to parasite on plants, which are varying in their form, life cycle and complexity in damaging to plants (Dey and Au, 1997). Sometimes toxic compounds are produced by some fungi and the products become inconsumable. Although plant breeders tend to produce resistant varieties, these pathogens soon adapt and they often overcome and break down such plant resistance (Aubertot et al., 2006). The importance of fungicides can be explained by reducing plant disease losses, increasing production in quantity and quality, and lowering hazards due to exposure to toxic substances produced by fungi in plant products (Ragsdale et al., 1991).

In spite of disadvantages that come with using chemicals in agricultural practices, chemical pesticides cannot be totally alternated with other management practices especially in commercial agriculture. However, restricted use of chemicals with high knowledge and care is of significant to reduce the environmental pollution and its related negative issues. Therefore, this study aimed to i) Evaluate of the efficiency of the fungicide Bentazole Combl 50% SL on pathogenic fungi Rhizoctonia solani and Fusarium solani ii) Test the effect of various doses of Bentazole Combl 50% SL and repeated treatments in the emergence of pesticide resistance in Rhizoctonia solani and Fusarium solani and iii) determine the minimum effective dose of the fungicide to control of diseases on cucumber caused by the two pathogenic fungi, to reduce the environmental pollution and to ovoid emergence of pesticide resistant strains.

Materials and Methods

The fungicide Bentazole Combl 50% SL (a abroad spectrum fungicide used to control various soil-borne fungi that cause plant diseases such as wilt and root rot on field crops, vegetables, fruits and nursery plants) used in the study was obtained from the Agricultural dealers in the province of Najaf.

Fungal isolates

The pathogenic isolates of Rhizoctonia solani and Fusarium solani were isolated from infected roots of cucumber plants. The infected root parts were washed with running water to remove adhesive soil particles, dried on the filter paper then cut into 1-2 cm parts and planted in Petri dishes containing sterilized P.D.A. medium. The dishes were incubated at 25 ± 2C°. After seven days of incubation, fungi were re-cultured and purified and then were diagnosed in the laboratory of plant pathology in the graduate division at faculty of agriculture/University of Kufa.

Culture media

Four different media were used in this study to culture, isolate, purify and diagnose pathogenic fungal isolates of Rhizoctonia solani and Fusarium solani. Media used were laboratory made Potato Dextrose Agar (PDA), Water Agar (WA), Commercial PDA as standard medium to differentiate some fungi and Potato Dextrose Broth (PDB).

Seed germination and Pathogenicity tests

The Radish seeds were subjected to evaluate seed viability and germination rate by placing 400 seed (in four 12cm Petri dishes 100 seed/dish) in paper towel moist with clean drinking water. The dishes were incubated for 72 hour at 30C°.

For the pathogenicity test, seeds were sterilized with 2% NaOCl for 2 min and then were washed with distilled water, socked with distilled water for 2 min tice. Seeds then were dyed using filter paper and cultured in Petri dishes containing WA medium already inoculated with either R. solani or F. solani and incubated for 48hour, and dishes were incubated. Seed germination rates were recorded at 7 days of incubation.

Effect of different doses of fungicide Bentazole Combl 50% SL on percent inhibition of radial growth of R.solani and F.solani

Pentazole Combl 50% SL was added to the sterilized PDA at four concentrations (four doses) 200, 100, 50 and 25% of the recommended dose to be 0.4, 0.2, 0.1 or 0.05 ml/L, in addition to the control (no fungicide). 20 ml from each medium type was poured in Petri dishes with three replicates/treatment and dishes were inoculated with 0.5 disk from 7 days old culture of either R. solani or F. solani . The dishes were then incubated at 25 ± 2 C °.Data were collected and percent of fungal growth stimulation/inhibition was calculated according to Abbot (1925) confirmed by Resistant resistance test Nurses R.solani and F. solani for chemical agent Bentazole Combl 50% SL on the plant medium P.D.B. After 30 days of cultivation of fungus Nurses R.solani and F.solani.

In this test, sterile PDB was distributed in 250 mL (200 ml each) conical flasks and 200 ml. The flasks were inoculated with three disks of F.solani or R.solani from 7 days old culture and the flasks were poisoned with the fungicide at doses and concentration described previously. Flasks were incubated at 25 ± 2 C ° for 30 days during which the flasks were agitated every three days period. After that, the fungal biomasses were extracted with sterile forceps, placed on filter paper to remove the free water. The fresh weight was recorded for each fungal biomass, and the biomasses were oven dry at 70 C° for 48 hours. The dry weight was recorded and values were subjected to calculate fungal growth stimulation/inhibition (Sha'ban and Al-Malah, 1993).

Statistical analysis

Results and Discussion

Seed germination and Pathogenicity tests

The results showed that the percentage of germination of radish seeds was 100% confirming a vitality of radish seeds used in the study.

Pathogenicity tests of R.solani and F.solani and their effects on the percentage of seed rot and eedlings death at seven days post planting on the W.A. medium at a temperature of 25 ± 2 C° (Table 1) showed that the highest percentage of rotting seeds was in the treatment of R.solani of F.solani (16.00 and 10.00%), respectively with significant differences compared to the control (0.00%). The highest infected rate resulted from R.solani (84.00%) compared to 0.00% infection from the control. The reason for the increase in the percentage of rotten seeds caused by R.solani, may be due to the nature of this fungus as it attacks the seeds of many plants families leading to seeds decomposition or preventing germination by secreting some toxic compounds that may affect or kill seed embryos and secretion of a number of enzymes for degrading cellulose, chitin and protein thus causing seed rot., and is one of the most important causes of seed rot and seed death In Iraq and the world (Rasmussen et al., 1989; Agrios, 2005; Bayati and Bahadli, 1988). It is also characterized by the secretion of enzymes and toxic compounds. Many of these compounds such as phenyl acetic acid and its derivatives have been diagnosed and purified (Dixon, 1993). In addition to many carbohydrate compounds containing glucose or manganese in their composition including glucose amine or N-acytyle glucose amine (Vidhyasekaran *et al.*, 1997). The fungus also secretes oxalic acid which kills plant cells and stimulate them to produce sugars and amino acids. R. solani is one of the fastest pathogens that kill their hosts. It was found that degrading enzymes such as Pectinase, Pectin methylledterase, Cellulase, and Phosphatase are found to be associated with fungus, (Murphy et al., 1984; Dillard, 1987).

Our findings showed that highest incidence of infection was by R.solani followed by F. solani. These findings are consistent with that reported by Saad (2001), Jabara (2002) and and Al-Malki (2002).

Table2. Effect of four different doses of the chemical pesticide Bentazole Combl 50% SL on R.solani radial growth stimulation/inhibition seven days on P.D.A. at 25 ± 2 C° for the first transfers
Values are means of three replicates. Number between parentheses is the percentage of the corresponding value.

Treatments	Fungal radial growth cm							Average
	Days							
	One	Two	Three	For	Five	Six	Seven	
``Control	1.30 (0.00)	3.58 (0.00)	6.43 (0.00)	8.73 (0.00)	9.00 (0.00)	9.00 (0.00)	9.00 (0.00)	6.72 (0.00)
Double dose	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)
Full dose	0.63 (51.53)	1.26 (64.80)	1.30 (79.78)	1.53 (82.37)	1.60 (82.22)	1.63 (81.88)	1.75 (80.55)	1.38 (74.73)
Half dose	0.22 (83.07)	1.38 (61.45)	1.65 (74.33)	2.18 (75.02)	2.53 (71.88)	2.96 (67.11)	3.50 (61.11)	2.06 (70.56)
Quarter dose	0.82 (36.92)	1.68 (53.07)	2.11 (67.18)	2.86 (67.23)	3.20 (64.44)	3.81 (57.66)	4.20 (53.33)	2.66 (57.11)
Average	0.59 (54.30)	1.58 (55.86)	2.29 (64.25)	3.06 (64.92)	3.26 (63.70)	3.48 (61.33)	3.69 (58.99)	
L.S.D.$_{0.05}$ dose		0.108	L.S.D.0.05 days		0.128	L.S.D.0.05 interaction		0.287

Effect of four different doses of the chemical pesticide Bentazole Combl 50% SL on R.solani radial growth stimulation/inhibition seven days on P.D.A. at 25 ± 2 C° for a series of two transfers

The first transfer of R. solani

The results for the effect of different doses (double dose, recommended dose, half dose, quarter dose and control) of pesticide Bentazole Combl 50% SL on growth of R. solani represented in table (2)showed the highest inhibitory rate at fourth day and the lowest on the first day, 64.92% and 54.30%,) respectively. For the treatments, significant differences were found in all the treatments compared to the control treatment. The results of interaction treatment of time and inhibition rate was the highest observed in the double dose (200%) treatment of Bentazole Combl 50% SL in all the testing days and the lowest rate of inhibition was in the control treatment for the all days.

It was also found (Table 2) that fungal radial growth increased as time increases. Complete inhibition (0.00 cm of radial growth) was in the double dose of the fungicide compared to radial growth of 6.72cm from the control treatment.

The second transfer of R.solani.

The results for the effect of different doses of pesticide Bentazole Combl 50% SL on growth of R. solani for the second transfer (cultured from fungal growth in the treatments of 25% of the recommended dose) as shown in table (2) showed the highest inhibitory rate was in the complete dose (100%) with inhibition rate of

88.22% compared to 0.00% inhibition from the control. For the treatments, significant differences were found in all the treatments compared to the control treatment. Results of interaction treatment of time and inhibition rate showed that highest inhibition rates were in the double dose followed by the complete recommended dose at day one and in the quarter dose at day seven compared to 0.00% inhibition from the control for the all testing days.

The results shown in Table (3) showed significant differences between the days compared to the first day. The highest radial growth occurred on day 7 and the lowest radial growth occurred on the first day, 5.60 and 1.62 cm, respectively. Radial growth was at the highest in the full dose of Bentazole Combl 50% SL reaching 8.15 cm compared to 1.03 cm from the control treatment. Interaction between treatments and time showed that the highest radial growth was in the control treatment in day 3-7 and the quarter dose treatment in the day 7 (9.00 cm) while the lowest radial growth (0.00 cm) was in the full dose and double dose of the fungicide treatments.

Table3. Effect of four different doses of the chemical pesticide Bentazole Combl 50% SL on R.solani radial growth stimulation/inhibition seven days on P.D.A. at 25 ± 2 C° for the second transfers

Treatments	Fungal radial growth cm – Days							Average
	One	Two	Three	For	Five	Six	Seven	
``Control	4.75 (0.00)	7.35 (0.00)	9.00 (0.00)	9.00 (0.00)	9.00 (0.00)	9.00 (0.00)	9.00 (0.00)	8.15 (0.00)
Double dose	0.00 (100.00)	0.66 (91.02)	0.95 (89.44)	1.20 (86.66)	1.41 (84.33)	1.65 (81.66)	1.76 (80.44)	1.09 (87.65)
Full dose	0.00 (100.00)	0.71 (90.34)	0.88 (90.22)	1.10 (87.77)	1.28 (85.77)	1.53 (83.00)	1.76 (80.44)	1.03 (88.22)
Half dose	1.36 (71.36)	1.96 (73.33)	2.86 (68.22)	3.23 (64.11)	3.45 (61.66)	4.00 (55.55)	4.90 (45.55)	3.10 (62.82)
Quarter dose	2.03 (57.26)	3.08 (58.09)	4.83 (64.33)	5.96 (33.77)	6.43 (28.55)	7.88 (12.44)	9.00 (0.00)	5.60 (36.34)
Average	1.62 (65.72)	2.75 (62.55)	3.70 (62.26)	4.09 (54.46)	4.31 (52.06)	4.81 (46.53)	5.28 (41.28)	
dose L.S.D.0.05		0.102	Days L.S.D.0.05		0.120	L.S.D.0.05 interaction		0.270

Values are means of three replicates. Number between parentheses is the percentage of the corresponding value.

Effect of four different doses of the chemical pesticide Bentazole Combl 50% SL on F.solani radial growth stimulation/inhibition seven days on P.D.A. at 25 ± 2 C° for a series of two transfers

The first transfer of F. solani

The study showed that there were significant differences in the percentage of inhibition between days compared to the first day (Table 4). The highest percentage of inhibition appeared on the first day and the lowest appeared on the seventh day (100.00 and 50.08% respectively). For the treatments, the highest percentage of inhibition occurred in the treatment of double dose of Bentazole Combl 50% SL (no fungal growth) and the lowest occurred in the control treatment (full fungal growth).

Table4. Effect of four different doses of the chemical pesticide Bentazole Combl 50% SL on F. solani radial growth stimulation/inhibition seven days on P.D.A. at 25 ± 2 C° for the first transfers

Treatments	Fungal radial growth cm							Average
	Days							
	One	Two	Three	For	Five	Six	Seven	
``Control	$0.00^{(0.00)}$	$0.97^{(0.00)}$	$1.70^{(0.00)}$	$2.90^{(0.00)}$	$3.90^{(0.00)}$	$4.15^{(0.00)}$	$4.62^{(0.00)}$	$2.60^{(0.00)}$
Double dose	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$
Full dose	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.95^{(75.64)}$	$1.61^{(61.20)}$	$1.45^{(68.61)}$	$0.57^{(86.49)}$
Half dose	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$0.00^{(100.00)}$	$1.11^{(71.53)}$	$1.41^{(66.02)}$	$1.75^{(62.12)}$	$0.61^{(85.66)}$
Quarter dose	$0.00^{(100.00)}$	$0.80^{(17.52)}$	$0.85^{(50.00)}$	$1.08^{(62.75)}$	$1.70^{(56.41)}$	$2.13^{(48.67)}$	$3.71^{(19.69)}$	$1.46^{(50.72)}$
Average	$0.00^{(100.00)}$	$0.35^{(63.50)}$	$0.51^{(70.00)}$	$0.79^{(72.55)}$	$1.53^{(60.71)}$	$1.86^{(55.17)}$	$2.30^{(50.08)}$	
dose L.S.D.$_{0.05}$		0.022	Days L.S.D.0.05		0.026	interaction L.S.D.$_{0.05}$		0.059

The results of interaction treatment of time and inhibition rate was the highest observed in the double dose (200%) treatment of Bentazole Combl 50% SL in all the testing days followed by the full dose for days 1-4 and the treatment of half dose for the same days and quarter dose for the first day only compared 0.00% inhibition from the control for all testing days and the lowest rate of inhibition was in the control treatment for all the testing days.

The second transfer of F. solani

Results showed that there were significant differences in the percentage of inhibition between days compared to the first day (Table 5). The highest percentage of inhibition was recorded in the first day and the lowest was in the seventh day, 67.20% and 47.87%, respectively. As for the treatments, the highest percentage of inhibition occurred in the treatment of double dose of Bentazole Combl 50% SL (90.10%) and the lowest occurred in the control treatment (0.00%). The results of interaction treatment of time and inhibition rate was the highest observed in the double dose (200%) treatment of Bentazole Combl 50% for the first, second and third days, and for the first day in the full dose and half dose treatments. However, the lowest inhibition rate was recorded in the control treatment for all the testing days.

Table5. Effect of four different doses of the chemical pesticide Bentazole Combl 50% SL on F. solani radial growth stimulation/inhibition seven days on P.D.A. at 25 ± 2 C° for the second transfers

Treatments	Fungal radial growth cm							Average
	Days							
	One	Two	Three	For	Five	Six	Seven	
``Control	1.25 (0.00)	2.32 (0.00)	3.60 (0.00)	4.47 (0.00)	5.42 (0.00)	6.60 (0.00)	7.57 (0.00)	4.46 (0.00)
Double dose	0.00 (100.00)	0.00 (100.00)	0.00 (100.00)	0.66 (85.23)	0.88 (83.76)	1.33 (79.84)	1.37 (81.90)	0.60 (90.10)
Full dose	0.00 (100.00)	0.20 (91.37)	0.71 (80.27)	0.91 (79.64)	1.48 (72.69)	1.75 (73.48)	1.91 (74.76)	0.99 (81.74)
Half dose	0.00 (100.00)	0.46 (80.17)	0.98 (72.77)	1.38 (69.12)	1.88 (65.31)	2.40 (63.63)	3.13 (58.65)	1.46 (72.80)
Quarter dose	0.80 (36.00)	1.30 (43.96)	2.28 (36.66)	3.73 (16.55)	4.20 (22.50)	5.00 (24.24)	5.75 (24.04)	3.29 (29.13)
Average	0.41 (67.20)	0.85 (63.10)	1.51 (57.94)	2.23 (50.10)	2.77 (48.85)	3.41 (48.23)	3.94 (47.87)	
dose L.S.D.$_{0.05}$		0.140	Days L.S.D.0.05		0.166	L.S.D.$_{0.05}$ interaction		0.327

Effect of different doses of Bentazole Combl 50% SL on biomass dry weight of R.solani and F.solani on P.D.B. at 25 ± 2C ° for 30 days of incubation

The study showed that percentage of inhibition (based on fungal biomass dry weight) was the highest in the case of F.solani and the lowest percentage of inhibition was in case of R.solani, 17.66% and 16.65%, respectively (Figure 1). In case of fungicide concentrations, the highest percentage of inhibition 30.85% occurred in the treatment of half the dose of Pentazole Combl 50% SL and the lowest percentage of inhibition (0.00%) was recorded in the control treatment. Interaction between the fungus species and pesticide concentrations showed that the highest percentage of inhibition

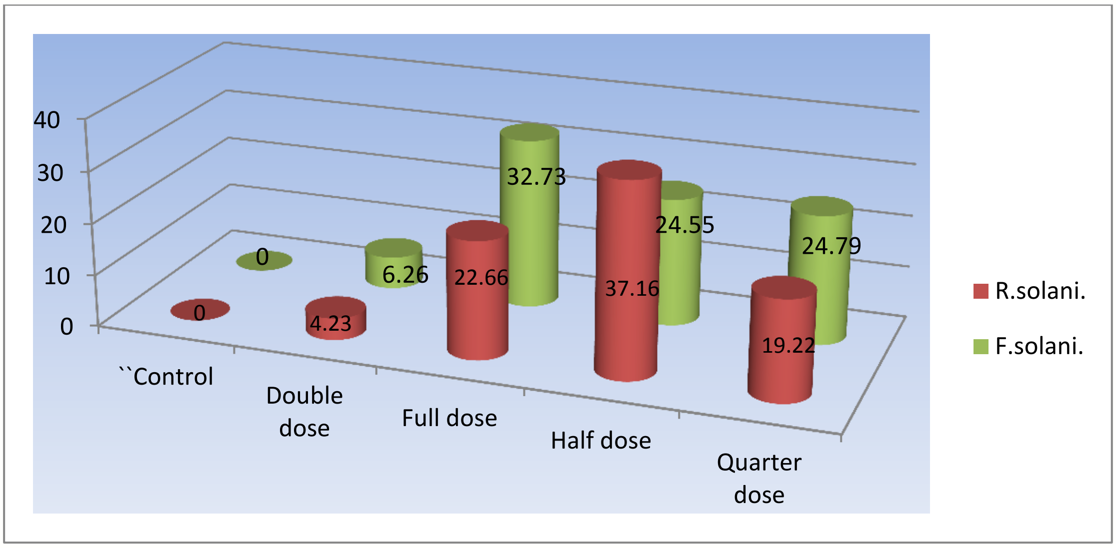

Figure1. Effect of four different doses of the chemical pesticide Bentazole Combl 50% SL on R. solani and F. solani biomass dry weight on P.D.B. at 25 ± 2 C° and 30 days of incubation

(37.16%) occurred in the treatment half of the recommended dose of Bentazole Combl 50% SL interacted with R.solani and the lowest percentage of inhibition occurred in control treatment with both R.solani and F.solani (0.00%).

Effect of different doses of Bentazole Combl 50% SL on biomass fresh weight of R.solani and F.solani on P.D.B. at 25 ± 2C ° for 30 days of incubation

(2) showed significant differences in the percentage of inhibition between the fungi and the highest percentage of inhibition was found in the treatment of fungus R.solani and the lowest percentage of inhibition appeared in the treatment of F.solani mushroom was (41.34 and 3.87) Respectively. For the concentration of the pesticide, the highest percentage of inhibition was observed in the full dose treatment (Pentazole Combl 50% SL) and the lowest percentage of inhibition in the control treatment was (37.33% and 0.00%) respectively. For the interaction between the fungus and pesticide concentrations where the highest percentage of inhibition occurred In the one-quarter dose dose, Bentazole Combl 50% SL + R.solani and the lowest percentage of inhibition occurred in the treatment of (R.solani and F.solani) reached (57.97 and 0.00%) respectively, and the interpretation of these results as abovem…..

The results obtained from the percentage of fungal radial growth inhibition of R..solani can be explained as the pesticide affects the fungal growth after two or three days post culturing in the poisonous medium (containing the pesticide). This is because the time period required for the fungicide pesticides to kill the fungus. (Aladil, 2006). It was noted that the effect of the pesticide gradually decreases by the

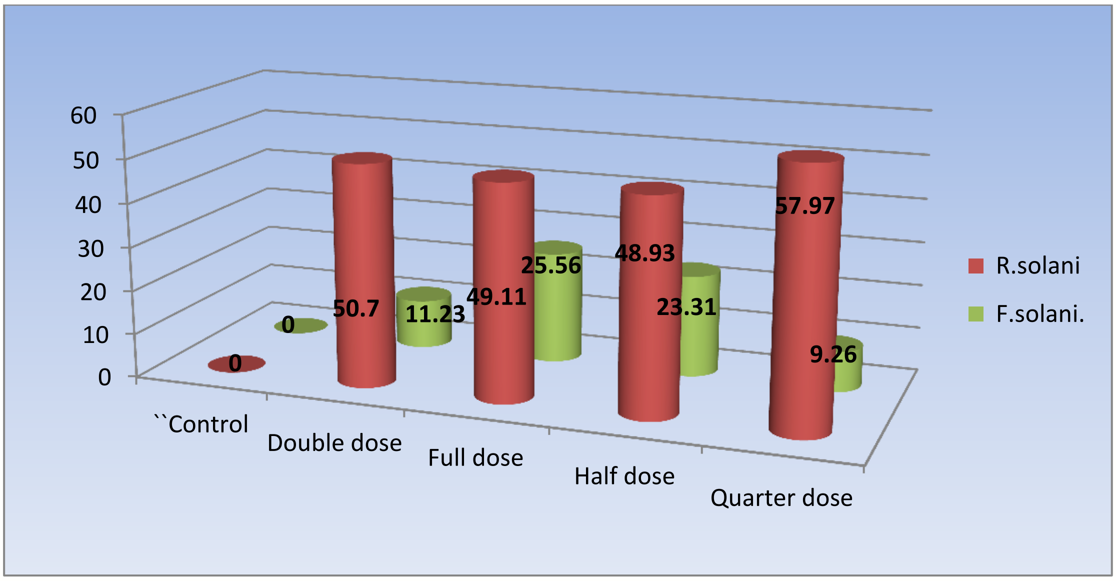

Figure2. Effect of four different doses of the chemical pesticide Bentazole Combl 50% SL on R. solani and F. solani biomass dry weight on P.D.B. at 25 ± 2 C° and 30 days of incubation

time progress, higher effect in the early days and then its effect is reduced until it is eliminated. This may be due to the ability of the fungus to breakdown the toxic substance and convert it into a non-toxic substance. The fungus metabolizes the pesticide either by secreting enzymes for pesticide metabolism and changing the composition partially to become less toxic than the original material or in the second part the toxic compound is converted into much higher polarity and then become more soluble in water thus can be removed out of the fungal body. Where most pesticides are low soluble in water and their oxidation or hydrolysis improvement helps to introduce polar groups to the combination to become more soluble to be more suitable to enter other reactions in what called primary metabolism. While the secondary metabolite happens when Bentazole Combl 50% SL is associated and liked with other compounds that have high molecular weight, such as Clucuronic acid, to form large molecules that are more soluble in water forming what called linked metabolism (Aladil, 2006)

The pesticide Bentazole Combl 50% SL has little effect but still sufficient to kill the fungus compared to control treatment. chemical pesticides can kill the organism by interfering with cellular respiration and energy production in cell mitochondria. Or by altering the bio-packaging of certain compounds which are vital for the of the organism, such as nucleic acids (RNA and DNA) and proteins. This affects the sugar glycolysis in cytoplasm and the Krebs cycle and oxidative phosphorylation in mitochondria (Aladil, 2006). This may explains the gradual reduced effect of the pesticide to be diminished by time as the fungus resist the pesticide effect by forming resistant compounds. This is in line with former studies that have suggested that phytophthora can resist pesticed Hymexazol (Toope et al. 2003)

The resistance may be acquired in the fungus as a result of not converting the pesticide into more effective substance and thus reducing toxicity. In the absence of the metabolic system, the organism may resist the compound (Aladil, 2006). It also explains that continuous exposure to chemicals increase the organism's tolerance and ability to break down such hydrocarbons, either by producing certain enzymes within the microorganism, inhibiting them, or by genetic changes that show new metabolic properties or convert them into less complex compounds (Cerniglia, 1997, Leahy and Colwell, 1990)

There are studies that suggest a range of possible explanations for the toxic effect of fungicides. First is the interaction of pesticides with biochemical enzymes, oxidation reactions. The second is the effects on biochemical compounds which affects the biological immunity to compounds needed by the fungal cell. Pesticide interaction with some amino acids affects protein synthesis (Sha'ban and Al-Mallah, 1993). This was confirmed by success of the fungal radial growth treated with low concentration of the fungicide especially the quarter dose of the recommended, where the small amount of active substance of the pesticide reaches the sensitive receptor sites to be ineffective (Fair, 2006).

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