

Induction of Systemic Resistance Against Root Rot of Basil Using Some Chemical Inducers

Mona M.M. Ragab*; M.M. Saber*; S.A. El-Morsy** and Abeer R.M. Abd El-Aziz**

* Fac. Agric., Cairo Univ., Giza, Egypt.

** Plant Pathol. Res. Inst., ARC, Giza, Egypt.

Some chemicals were evaluated for inducing systemic resistance against root rot in basil. Fungi belonging to six genera were isolated from diseased plants. The survey showed differences in the frequency of the isolated fungi. In general, *Rhizoctonia solani* was found to be the most frequently isolated fungus. Percentage of pre- and post-emergence damping-off caused by *Rhizoctonia solani* was decreased by using all tested chemicals compared to check treatment. The growth regulators Oxalic acid (4 mM), potassium chloride (4%), indole acetic acid (400ppm) were superior treatments for reducing root rot incidence under greenhouse conditions. Considerable increases in activity of oxidative reductive enzymes (peroxidase and polyphenoloxidase) and chitinase enzyme were recorded in plants grown from treated basil seeds. Salicylic acid, oxalic acid, potassium chloride (KCl) and indole acetic acid (IAA) were the superior treatments in this respect.

Keywords: Chitinase, induced resistance, peroxidase, polyphenoloxidase, *Rhizoctonia solani*, root rot and sweet basil

Sweet basil (*Ocimum basilicum* L.) is one of the aromatic plants that have a major role in agriculture and industry. They are the main source for safety drugs and raw substances used in manufacturing of pharmaceuticals. Fungi belonging to various genera were isolated from infected plants. *Rhizoctonia solani* was the main causal of root rot of basil plants as it recorded the highest frequency (Garniel *et al.*, 1996 and Chiocchetti *et al.*, 2001).

Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of the induced resistance; in both SAR and ISR, plant defences are preconditioned by prior infection or treatment that results in resistance (or tolerance) against subsequent challenge by a pathogen. Great strides have been made over the past 20 year in understanding the physiological and biochemical basis of SAR and ISR. Much of this knowledge is due to the identification of a number of chemical and biological elicitors; some of which are commercially available for use in conventional agriculture (Vallad and Goodman, 2004). Several investigators studied the effectiveness of these chemical inducers on root rot disease (Segarra *et al.*, 2006). The induced systemic resistance (ISR) sensitizes the plant to respond rapidly after treatment. These responses include phytoalexin accumulation, phenol, lignifications and activation of peroxidase, polyphenoloxidase and chitinase.

This research aimed to study the effect of some chemical inducers in controlling root rot in basil and to identify some associated biochemical changes in treated plants.

Materials and Methods

1- Disease survey of basil root rot in different governorates:

Survey was carried out in three different governorates in Egypt namely Assiut, Beni-Suef and Menia during the two successive seasons of 2006/2007 and 2007/2008. Basil diseased plants showing root rot symptoms were collected from basil growing fields and greenhouses. The average percentage of disease incidence was calculated as the number of rotted basil plants in relative to the total number of examined plants.

2- Isolation, purification and identification of the fungi associated with basil diseased plants:

Infected roots and stems were cut into small fragments, washed thoroughly with tap water, then sterilized with sodium hypochloride solution (1%), then dried between two sterilized filter papers. Fragments were then placed on potato dextrose agar media in Petri dishes and incubated at $27\pm 1^{\circ}\text{C}$ for 7 days. Developed fungal colonies were purified using either hyphal tip or single spore techniques. The isolated fungi were picked from the edges of growing colonies and transferred onto water agar plates using the methods suggested by Nelson *et al.* (1983) and Booth (1971) and the purified colonies were then transferred to PDA slants. All the obtained isolates were microscopically identified according to the morphological features using the description of Barentt and Hunter (1972) and Ellis (1971). Identification of the selected isolates was confirmed by the Fungal Taxonomy Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza.

3- Pathogenicity tests:

Fungal inocula were prepared by inoculating maize meal-sand medium in 500 ml glass bottles with 5mm. disk of a 7-day-old culture of the pathogen tested then incubated at $27\pm 1^{\circ}\text{C}$ for 15 days. Throughout all greenhouse trials, pots of 25 cm in diameter were used. Pots were always sterilized by immersing in 5% formalin for 15 minutes and then air dried for 5 days. Soil infestation was carried out by adding the fungal inoculum to the sterilized soil at the rate of 3% of soil weight (Mazen, 2004). Fungal inocula were thoroughly mixed with the soil and regularly watered every day for a week before planting to ensure even distribution and growth of each particular fungus. Soil mixed alone with the same amount of autoclaved maize meal-sand medium served as a check treatment. Each replicate pot was planted by five seeds of basil and four pots were used for each treatment. All plants were observed daily and the pre-and post- emergence root rot was recorded after 15 and 30 days of sowing, then the survived plants were counted according to the following formula:

$$\text{Pre-emergence (\%)} = \frac{\text{Total No. of ungerminated seeds}}{\text{Total No. of planted seeds}} \times 100$$

Post-emergence (%) = $\frac{\text{Total No. of rotted seedlings}}{\text{Total No. of planted seeds}} \times 100$

Survived seedlings (%) = $\frac{\text{Total No. of survived seedlings}}{\text{Total no. of planted seeds}} \times 100$

4- *Effect of soaking basil seeds in some chemical inducers agents on controlling root rot under greenhouse conditions:*

Basil seeds were soaked in the solutions of each tested chemical for 2.5 hr (Khaleifa *et al.*, 2007) before planting. Each particular treatment consisted of three different concentrations (potassium chloride, dipotassium phosphate and disodium phosphate were used at 1, 2 and 4 % , indole butyric acid and indole acetic acid were used at 100, 200 and 400 ppm., tannic acid, oxalic acid and salicylic acid were used at 2, 4 and 8 mM). The wetted seeds were spread in a thin layer and left about 24 hours, then sown in the infested potted soil with the virulent *R. solani* isolate (R1A), at the rate of 5 seeds/pot. Seeds soaked in tap water were sown in pots to serve as check. Three pots for each treatment were used as replicates (Mazen, 2004). Pre- and post- emergence root rot incidence were recorded after 15 and 30 days of sowing. The survival of basil plants was also recorded after 60 days from sowing.

5- *Effect of some chemical inducers on the biochemical changes of basil plants grown in soil infested by R. solani:*

After 15 days from planting, fresh samples were taken from plants grown from previously treated and untreated basil seeds and extracted according to Goldschmidt *et al.* (1968). Then the extracts were used for assaying biochemical change associated with the tested treatments of chemical inducers, the activities of peroxidase enzyme (Allam and Hollis, 1972) and polyphenoloxidase enzyme (Snell and Snell, 1953) and chitinase enzyme (Tuzun *et al.*, 1989) were determined.

6- *Statistical analysis:*

The obtained data were statistically treated by analysis of variance (ANOVA) using the Fisher LSD method. Means were separated by Fisher's protected least significant differences (LSD) at $P \leq 0.05$ level (Gomez and Gomez, 1984).

Results

1- *Survey of basil root rot in different governorates*

Data presented in Table (1) show that mean percentages of natural disease incidence ranged between 12.8 and 17.1% within governorates, whereas they were 14.96 and 15.20% during the two seasons, respectively. However, the highest percentage of infection (18.0%) was recorded in Assiut governorate in the first season (2006/2007), while the least (12.2%) was obtained from Beni-Sweif in the same season.

2- *Isolation, purification and identification of the fungi associated with basil diseased plants:*

Data presented in Table (2) indicate that fungi belonging to six genera were isolated from root and stem parts of naturally infected basil plants. However, *Rhizoctonia solani* was the most frequently (23.12%) isolated fungus.

Table 1. Mean percentage of natural infection by root rot disease of basil plants at different governorates during 2006/2007 and 2007/2008 growing seasons

Governorate	Mean percentage of natural infection/season		
	2006/2007	2007/2008	Mean
Beni-Sweif	12.2	13.5	12.8
Menia	14.7	15.9	15.3
Assiut	18.0	16.2	17.1
Mean	14.9	15.2	--

Table 2. Fungi isolated from basil plants showing root rot symptoms and their frequency

Isolated fungi	No. of isolates	Frequency (%)
<i>Alternaria</i> spp.	13	4.06
<i>Fusarium moniliforme</i> Sheldon	28	8.75
<i>Fusarium oxysporum</i> (Schlect. Emend Snyd.& Hans)	65	20.31
<i>Fusarium solani</i> (Mart.) Apple & Wr.Emend. Snyd.&Hans)	32	10.00
<i>Macrophomina phaseolina</i> (Maubl.) Ashby	67	20.94
<i>Penicillium</i> spp.	17	5.31
<i>Pythium</i> spp.	24	7.51
<i>Rhizoctonia solani</i> Kuhn	74	23.12
Total	320	100.00

3-Pathogenicity tests:

Pathogenicity of 9 isolates of *Rhizoctonia solani*, chosen to represent all the surveyed three Egyptian governorates, was evaluated on basil plants under favourable greenhouse conditions. Data in Table (3) show that all tested isolates of *Rhizoctonia solani* were pathogenic, at different degrees, on the tested basil plants. Data indicate that isolate No. R1A (from Assiut) caused the highest percentage of pre- and post-emergence root rot (43.3 and 26.0%, respectively) on the other hand, the lowest virulent isolate (R1E) was obtained from Menia governorate.

4- Effect of soaking basil seeds in some chemical inducers agents on controlling root rot under greenhouse conditions:

4.1. Effect of some antioxidants:

Data presented in Table (4) show the effect of salicylic acid and oxalic acid at the used concentrations on the percentages of pre- and post- emergence damping off

Table 3. Pathogenicity of *R. solani* isolates on basil under greenhouse conditions

Source of isolates (Governorate)	Isolate No.	Pr-emergence (%) (after 15 days)	Post-emergence (%) (after 30 days)	Plant survival (%) (after 60 days)
Beni-Sweif	R1B	33.3	20.0	46.7
	R2B	33.3	23.3	43.4
	R3B	26.6	10.0	63.4
Menia	R1E	13.3	20.0	66.7
	R2E	31.3	20.3	48.4
	R3E	33.3	23.3	43.4
Assiut	R1A	43.3	26.0	30.7
	R2A	35.0	20.3	44.7
	R3A	40.0	20.0	40.0
Check		4.3	0.0	95.7
L.S.D at 5%		2.75	2.08	3.35

caused by *R. solani* was significant compared to control. However, oxalic acid was the most effective inducer for decreasing percentage of pre- and post- emergence root rot, being 12.4 and 8.9% followed by salicylic acid being 16.5 and 11.6 % on the average, respectively without significant differences. On the other hand, ascorbic acid showed no effect (26.1 and 14.5%), oxalic acid caused the highest percentages of plant survival (78.7%) compared with the check treatment (58.4%).

Table 4. Effect of soaking basil seeds in the solutions of some antioxidants on percentages root rot incidence in soil infested with *R. solani* under greenhouse conditions

T	Pre-emergence (%)				Post-emergence (%)				Plant survival (%)			
	2 mM	4 mM	8 mM	Mean	2 mM	4 mM	8 mM	Mean	2 mM	4 mM	8 mM	Mean
SA	22.5	16.3	10.8	16.5	14.2	11.1	9.6	11.6	63.3	72.6	79.6	71.9
OA	15.3	12.6	9.3	12.4	14.2	7.9	4.8	8.9	70.5	79.5	85.9	78.7
AA	32.3	26.9	19.1	26.1	16.5	14.1	12.9	14.5	51.2	59.0	68.0	59.4
C	25.0	25.0	25.0	25.0	16.6	16.6	16.6	16.6	58.4	58.4	58.4	58.4
LSD 5%	Inducers (I) =2.13				3.11				5.28			
	Concentrations(c) =2.52				2.14				3.04			
	DXC =5.19				5.11				8.32			

Whereas: T= Treatment, SA= Salicylic acid, OA= Oxalic acid, AA= Ascorbic acid and C= Check treatment

4.2. *Effect of some salts*

Data presented in Table (5) show clearly that soaking the basil seeds in any of the solutions of the three salts significantly decreased the pre- emergence damping-off. However, potassium chloride (KCl) was the most effective inducer at 4% for decreasing percentages of pre-, and post- emergence root rot, being 10.5 and 5.6%, respectively. Meanwhile, disodium phosphate (Na_2HPO_4) gave the least effect as 12.6 and 10.4%, respectively.

Table 5. Effect of soaking basil seeds in the solutions of some salts on percentages of pre- and post-emergence root rot incidence in soil infested with *R. solani* under greenhouse conditions

T	Pre-emergence (%)				Post-emergence (%)				Plant survival (%)			
	1%	2%	4%	Mean	1%	2%	4%	Mean	1%	2%	4%	Mean
KCl	16.5	14.2	10.5	13.7	10.5	8.4	5.6	8.2	73.0	77.4	83.9	78.1
K_2HPO_4	16.9	15.7	11.0	14.5	9.5	8.3	6.5	8.1	73.6	76.0	82.5	77.4
Na_2HPO_4	18.5	16.0	12.6	15.7	14.6	11.6	10.4	12.2	66.9	72.4	77.0	72.1
Check	25.0	25.0	25.0	25.0	16.6	16.6	16.6	16.6	58.4	58.4	58.4	58.4
LSD 5%	Inducers (I) =2.17 Concentrations (c) =2.01 IXC =4.31				2.74 1.95 3.66				3.21 2.91 5.17			

Whereas: T= Treatment.

4.3. *Effect of some growth regulators*

Data in Table (6) indicate that soaking basil seeds in the solutions of some growth regulators decreased significantly the pre- and post- damping off and increased plant survival show that indole acetic acid (IAA) at 400 ppm gave the highest effect on the percentages of pre- and post- emergency damping off (8.2 and 5.3%) followed by indole butyric acid (IBA).

5. *Effect of soaking basil seeds in solutions of some chemical inducers on the biochemical changes of basil plants in soil infested with *R. solani*:*5.1. *Effect on peroxidase and polyphenoloxidase activity*

Data presented in Table (7) indicate that basil plants grown from seeds soaked in the solutions of different inducers resulted in an increase of peroxidase and polyphenoloxidase activity compared to the untreated control. Oxalic acid 8 mM gave the highest increment in peroxidase activity (3.94 activity/min) followed by (IAA) 400 ppm indole acetic acid (3.74 activity/min). Meanwhile, 8 mM ascorbic acid gave the least (2.18 activity/min) compared to check treatment (1.42 activity/min). Furthermore, oxalic acid gave the highest increasing in polyphenoloxidase activity followed by potassium chloride (KCl) and IAA, being 1.99, 1.98 and 1.87 activity/min, respectively. Meanwhile, ascorbic acid gave the lowest value (1.05 activity/min) compared to check treatment (0.9) activity/min).

Table 6. Effect of some growth regulators as seed soaking on percentages of pre, post emergence and plants survival in soil infested by *R. solani* under greenhouse conditions

T	Pre-emergence (%)				Post-emergence (%)				Plant survival %			
	100 ppm	200 ppm	400 ppm	Mean	100 ppm	200 ppm	400 ppm	Mean	100 ppm	200 ppm	400 ppm	Mean
IAA	12.5	10.0	8.2	10.2	11.0	9.5	5.3	8.6	76.5	80.5	86.5	81.2
IBA	14.6	11.9	9.5	12.0	12.0	9.5	6.5	9.3	73.4	78.6	84.0	78.7
Check	25.0	25.0	25.0	25.0	16.6	16.6	16.6	16.6	58.4	58.4	58.4	58.4
L.S.D at 5%	Inducers (l)=1.17 Concentrations (c)=2.01 IXC =4.31				2.11 1.78 3.68				2.71 1.39 3.54			

Whereas: T= Treatment, IAA= Indole acetic acid, IBA= Indole butyric acid.

Table 7. Determination of peroxidase and polyphenoloxidase activity (activity/min) in basil plants grown from seeds treated with different chemical inducers and planted in soil infested with *R. solani*

Treatment	Concen.	Peroxidase activity activity/min*	% to control	Polyphenoloxidase activity activity/min*	% to control
KCl	4%	2.98	209	1.98	217
K ₂ PO ₄	4%	2.76	194	1.30	142
Na ₂ PO ₄	4%	2.51	167	1.22	134
Salicylic acid	8mM	3.30	232	1.63	179
Oxalic acid	8mM	3.94	277	1.99	218
Ascorbic acid	8mM	2.18	153	1.05	115
IAA	400ppm	3.74	263	1.87	205
IBA	400ppm	3.21	226	1.55	170
Check	-		1.42		0.91

* Enzyme activity is expressed as change in absorbance/minute /g fresh weight

5.2. Chitinase enzyme activity:

Data in Table (8) recorded that treatment of basil seeds with different chemical inducers resulted in an increase in chitinase activity compared to the untreated Check. KCl gave the highest increasing (6.64 activity/min) followed by SA (5.85 activity/min) while ascorbic acid gave the least (2.95 activity/min) compared to other treatments.

Table 8. Determination of chitinase activity (min/activity) in basil plants grown from seeds soaked in the solutions of some chemical inducer and planted in soil infested with *R. solani* under greenhouse conditions

Treatment	Concentration	Chitinase/min activity
KCl	4%	6.64
K ₂ HPO ₄	4%	4.11
Na ₂ HPO ₄	4%	3.96
Salicylic acid	8mM	5.85
Oxalic acid	8mM	5.12
Ascorbic acid	8mM	2.95
IAA	400ppm	5.24
IBA	400ppm	5.21
Check	-	2.31

Discussion

Sweet basil (*Ocimum basilicum L.*) is one of the most important medicinal and aromatic plants. Soil borne diseases including wilt and root rot cause important considerable losses in yield. In the present investigation, extensive survey was conducted throughout three Egyptian governorates to determine the occurrence and frequency of various fungi associated with diseased basil plants. Fungi belonging to six genera were isolated from diseased plants. The survey showed differences in the frequency of the isolated fungi. In general, *Rhizoctonia solani* was found to be the most frequently isolated fungus. These results are in harmony with those reported by other researchers (Hilal *et al.*, 1998 and Chiocchetti *et al.*, 2001).

Pathogenicity test of nine isolates of the most frequently isolated fungus, *Rhizoctonia solani*, proved that they were pathogenic and virulent for basil. Percentages of pre- and post- emergence root rot were varied according to the fungus isolate. Isolate No.R1A (from Assiut) caused the highest percentage of pre- and post-emergence root rot incidence recorded as 43.3% and 26.0%, respectively, while the lowest was caused by isolate No. R1E from Menia governorate.

Induced systemic resistance (ISR) of plants against pathogens is a widespread phenomenon that has been investigated with respect to the underlying signalling pathways as well as to its potential use in plant protection. Elicited by a local infection, plants respond with a salicylic acid dependent signalling cascade that leads to the systemic expression of a broad spectrum and long-lasting disease resistance that is efficient against fungi, bacteria and viruses (Heil and Bostock, 2002).

The tested chemical inducers might stimulate some defence mechanisms such as phenolic compounds, oxidative enzymes and some metabolites. In the present work, the activity of peroxides and polyphenoloxidase and chitinase enzymes was obviously higher in plants grown from treated seed compared to the untreated. In

general, oxalic acid, SA and KCl were the most effective for stimulating these defence mechanisms. The present results are in agreement with those recorded by Ibrahim (2006).

Oxidative-reduction enzymes play an important role in induced resistance by the oxidation of phenols to oxidized products (Quinine) which limit the fungal activity (Shalaby *et al.*, 2001 and Hassan *et al.*, 2007). Peroxidase also catalyzes the final polymerization step of lignin synthesis, and may therefore be directly associated with the increased ability of systemically protected tissue to lignify which may restrict the penetration (Gross, 1979). The increase in PO activity has an important function in secondary cell wall biosynthesis by polymerizing hydroxyl and methylhydroxy cinnamic alcohols into lignin and forming rigid cross-links between cellulose, pectin hydroxyproline in glycoproteins (HPLGP) and lignin (Grisebach, 1981). Polyphenoloxidase is a widespread enzyme found in plant cells located in the chloroplast membranes. This enzyme is capable of dehydrogenating of O-diphenols to produce O-quinones. However, it indicates the highest activity toward hydroxylation of monophenols to diphenols. Oxidation of phenolic compounds in plant cells is responsible for initiating the browning reaction of the tissues and is identified as presence of the pathogenic factor. Moreover, Polyphenoloxidase induces metabolization of these phenolic compounds into toxic forms. (Chranowski *et al.*, 2003). Chitinase activities may be involved in the defence of plants against fungi and bacteria by their action on the cell walls of invading pathogens. Several investigators have indicated that induced resistance in plants is associated with a great increase in chitinase activities (Metraux and Boller, 1986; and Vallad and Goodman, 2004).

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(Received 22/03/2009;
in revised form 27/05/2009)

استحثاث المقاومة الجهازية في نباتات الريحان ضد مرض عفن الجذور باستخدام بعض الكيماويات

منى محمود ماهر رجب*، مجدى محمد صابر*،
سامى عبد الفتاح المرسي** وعيبر رمضان محمد عبد العزيز**
* كلية الزراعة- جامعة القاهرة- الجيزة- مصر
** معهد بحوث امراض النباتات- مركز البحوث الزراعية- الجيزة.

يعتبر نبات الريحان من النباتات العطرية التي لها فوائد طبية متعددة وهو من النباتات التي تصدر الى عديد من الدول مثل ألمانيا وإيطاليا وهولندا والولايات المتحدة. اشتمل هذا البحث على اجراء حصر لمرض عفن الجذور في الريحان وذلك في ثلاث محافظات هي بنى سويف وأسيوط والمنيا، وعزل الفطريات المسببة له وثبت من الحصر الذى اجرى خلال موسمى ٢٠٠٦/٢٠٠٧ - ٢٠٠٧/٢٠٠٨ ان ١٤.٦٩ الى ١٥.٢٠ % على التوالي من النباتات التي تم فحصها ظهرت عليها أعراض الإصابة بأعفان الجذور. تم عزل عدد من الفطريات التي تتبع ستة أجناس فطرية من النباتات المصابة ، حيث كان الفطر ريزوكتونيا سولاني هو الأكثر تكرارا . تبين من اختبارات القدرة المرضية أن العزلة RIA المعزولة من محافظة أسيوط هي الأعلى في القدرة المرضية بينما العزلة RIE المعزولة من محافظة المنيا كانت الأقل في القدرة المرضية.

وفي تلك الدراسة تم نقع بذور الريحان قبل الزراعة في تركيبات مختلفة لبعض المواد المحفزة لآليات الدفاع الطبيعية وهي حامض الساليسليك و حامض الأوكساليك و حامض الأسكوربيك بتركيزات ٨،٤،٢ ملليمول واستخدام بعض الأملاح مثل كلوريد البوتاسيوم وفوسفات البوتاسيوم وفوسفات الصوديوم بتركيزات ١% ، ٢% ، ٤% واستخدام بعض منظمات النمو مثل الأندول بيوتريك أسيد و أندول أستيك أسيد بتركيزات ١٠٠، ٢٠٠، ٤٠٠ جزء في المليون وذلك ضد مرض عفن جذور الريحان المتسبب عن الفطر ريزوكتونيا سولاني تحت ظروف الصوبة . أظهرت جميع المواد المستحثة فعالية كبيرة في نسبة خفض الإصابة بعفن الجذور وموت اللبانات تحت وفوق سطح التربة وكذلك انخفاض الإصابة بمرض عفن الجذور وزيادة نسبة النباتات السليمة مقارنة بالنباتات غير المعاملة بهذه المواد وكانت معاملات حامض الأوكساليك بتركيز ٨ ملليمول وكلوريد البوتاسيوم بتركيز ٤% و حمض الأندول أستيك أسيد بتركيز ٤٠٠ جزء في المليون هي أفضل المعاملات للمختبرة في خفض نسبة الإصابة بمرض عفن الجذور وزيادة نسبة النباتات السليمة .

وقد أظهرت دراسة التغيرات الكيميائية الحيوية كمؤشرات عن المقاومة المستحثة زيادة كبيرة في نشاط انزيمات الاكسدة والاختزال مثل البيروكسيداز والبولي فينول لوكسيداز وانزيم الشيتيناز، وكانت معاملات حمض الأوكساليك وحمض الساليسليك وكلوريد البوتاسيوم والأندول أستيك أسيد هي أفضل المعاملات للمختبرة في هذا المجال مقارنة بالنباتات غير المعاملة بينما ملح فوسفات الصوديوم للتثاقية وحمض الأسكوربيك أقل تأثيرا .