

## Effectiveness of some Plant Extracts as Safe Control Means against Damping-off and Root Rot Diseases in Canola Plants

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Received: 21/4/2021

**Abstract:** Canola damping-off diseases root rot and wilt are considered the most serious diseases that cause considerable losses in canola yeild and quality the causal organisms were isolated from infected root sampeles identified as *Fusarium solani* (Sacc), *Rhizoctonia solani* (Kühn) and *Fusarium oxysporum* (Schlecht). In this study, aqueous extracts and ethanol solvent extracts of four wild medicinal plants (*Moringa olifera*, *Ocimum basilicum*, *Cinnamomum camphora* and *Lantana camara*) were evaluated *in vitro* and under greenhouse for protection of canola plants against root rot and wilt diseases. *In vitro*, all aqueous and ethanol solvent extracts significantly suppressed linear growth of *F. Solani*, *R. solani* and *F. oxysporum* with different degrees. *Moringa olifera*, was superior the mycelial growth of all tested fungi. Also, ethanol solvent was more active than aqueous extracts of all tested plants. Under greenhouse conditions, all tested extracts significantly reduced damping-off, root rot and wilt incidence compared with untreated control. Canola plants treated with *Moringa olifera* extracts gave the highest protection against damping-off, root rot and wilt compared to the other tested extracts. These results suggested that aqueous and ethanol solvent extracts may play an important role for controlling canola root rot and wilt diseases.

**Keywords:** Canola, damping-off, root rot, wilt disease, plant extracts, *Moringa olifera*, Basil, Camphor, *Lantana camara*

### INTRODUCTION

Canola (*Brassica napus* L.) is an important oil seed crops in most countries of the world and one of the promising oil crop in Egypt characterized with high oil content and ability to cultivate in newly reclaimed sandy land under moderate stress conditions (Haag *et al.*, 1983; Silveira, 1984; Safiullina, 1984.).

Root-rot and wilt disease of canola is a soil-borne disease that is incited by several fungal pathogens including *Fusarium* spp., *Rhizoctonia solani*, *Pythium* sp. and *Macrophomina phaseolina*. The pathogens are known to be very persistent in soil and capable of surviving in infested soil and resulted in seed rot, damping-off, crown rot, root and pod rot (Melzer *et al.*, 2016). Root rot also reduces the ability of the canola root system to uptake nutrients and water from soil particularly in the late stage of disease process, thereby reducing the quantity and quality of canola seed yield and shorting the growing season. EL-Korashy (1997) reported that *Datura* extracts was the most effective against *Rhizoctonia solani*, *Fusarium solani* and *Sclerotium rolfsii* followed by Spairment while Neem and Common basil had moderate effect. Adandonon *et al.* (2006) found that *Moringa* seed treatment combined with *Trichoderma* soil sprinkle resulted in significantly more than 94% and 70% disease control damping off in the greenhouse and field, respectively, with significant yield increase in the cowpea field. Anju *et al.* (2008) found that *T. harzianum* with 10 and 50% Neem leaf extract resulted in 100% inhibition of the growth of the pathogens *Alternaria solani*, *Fusarium oxysporum* and *Colletotricum gloeosporioides* [*Glomerella cingulata*] *in vitro*. EL Morsy *et al.* (2011) found that organic solvent extracts of lemon grass leaves were more effective than those of rhubarb roots in inhibiting mycelial growth of *F. solani* and *R. solani*. Farag *et al.* (2011) said that neem and willow aqueous extracts reduced the disease incidence of

*Fusarium* wilt in tomato seedlings by increasing the activities of antioxidant defensive enzymes and decreasing the level of lipid peroxidation. Aye and Matsumoto (2011) found that cloves, Neem leaf, Rosemary and Pelargonium are potential phytoextracts to control *R. solani*. Seema *et al.* (2011) found that the antifungal effect of 10 plant extracts viz., *Thevetia peruviana*, *Ocimum basilicum*, *Piper betel*, *Murraya koenigii*, *Chrysanthemum coronarium*, *Polyalthia longifolia*, *Catharanthus roseus*, *Pelargonium graveolens*, *Moringa officinalis* and *Lawsonia inermis* were evaluated by poisoned food technique against *Rhizoctonia solani* only four plants namely, *Lawsonia inermis*, *Piper betel*, *Polyalthia longifolia* and *Pelargonium graveolens* have recorded significant antifungal activity. Seint and Masaru (2011) found that all of *Rhizoctonia solani*, *Rhizoctonia oryzae*, *Rhizoctonia oryzae-sativae* and *Sclerotium hydrophilum* fungal growths were suppressed 100% by using clove extract. Neem leaf, rosemary and pelargonium extracts were found to give the second best suppression against the tested fungi. Neem leaf extract inhibited the growth of *R. solani* by 87.5%, *R. oryzae* by 92.5% and *R. oryzae-sativae* by 80%.

However, the same extract inhibited *S. hydrophilum* by only 49.1%. Rosemary extract gave an inhibition of 67.7% for *R. solani*, 88.0% for *R. oryzae*, 86.0% for *R. oryzae sativae* and 73.89% for *S. hydrophilum* Lakpale (2013) found that leaf extract of *Tulsi*, *Amahaldi* and *Pudina* against *R. solani*; *Tikhur*, *Jangali-onion* and *Neem* against *S. rolfsii* and *Neem*, *Tulsi* and *Datura* against *M. phaseolina*; botanicals like *Achook* and *Neem-azal* against *R. solani*; *Neem-azal* and *Wanis* against *S. rolfsii* and *Achook* and *Biotos* against *M. phaseolina* were found very effective. Dissanayake and Jayasinghe (2013) found that methanol extracts from sweet flag shows a marked effect of the 20% with inhibition values of 91%, 86%

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and 84% for *F. oxysporum*, *R. solani* and *C. mucea* whereas those from wild basil inhibited the growth of the same pathogens by 89%, 84% and 74%. Farzana *et al.* (2014) found that the antifungal activity of ethanol and acetone extract of leaves of nine medicinal plants: *Piper betel*, *Lowsonia inermis*, *Psidium guajava*, *Carica papaya*, *Moringa oleifera*, *Mimosa pudica*, *Catharanthus roseus*, *Adhatoda vasica* and *Andrographis paniculata* against *Fusarium oxysporum* was assessed.

All the extracts inhibited mycelial growth at various levels. Among them the superior inhibition (100%) was found in 15% concentration of ethanol extract of *Lowsonia inermis* and *Psidium guajava* against *Fusarium*. Priyanka *et al.* (2014) found that the extract of *Ageratum conyzoides* exhibited maximum toxicity (95.57%) against the *Fusarium oxysporum f. sp. lycopersici*. Significant results were also observed in extracts of *Ageratum haustonianum*, *Clerodendrum inermeae* and *Terminalia bellirica* showing inhibition of 90.33%, 84.97% and 79.19%, respectively. Bhumika *et al.* (2014) found that thirteen Plants leaf extracts were tested *in vitro* for their potential to control *Rhizoctonia solani*. Garlic, eucalyptus, lemongrass, *Gokhru* and *Van tulsi* significantly inhibited the mycelial growth and sclerotial production except *Tulsi*, Onion, *Aak*, *Jatropha*, *Beshram* failed to inhibit the mycelial growth of *Rhizoctonia solani* Nupur and Rashmi. (2015) found that the efficacy of 13 plant extracts viz-leaf extracts of *Azadirachta indica*, *Catharanthus roseus*, *Lantana camara*, *Ocimum sanctum* *Ricinus communis*, *Saraca indica* and *Thuja occidentalis*, latex yielding plants *Calotropis procera*, *Nerium indicum*, *Datura*, *Ficus religiosa* and bulbs of *Allium cepa* and *Allium sativum* were tried to control root rot pathogen *in vitro*. They were evaluated for their antifungal activity over *Rhizoctonia solani*. All the plant extracts showed significant reduction in the growth of the pathogen compared with control.

Abdurrahman and Hayriye (2016) found that methanol extracts of different parts (leave, flower, root, fruit and shoots) of *Trachystemon orientalis*, *Smilax excelsa*, *Rhododendron ponticum*, *Phytolacca americana* and *Prunus laurocerasus*, showed significantly distinguished antifungal activities against *Alternaria solani*, *Botrytis cinerea* and *Rhizoctonia solani*. Goss *et al.* (2017) said that Moringa leaf and seed extracts contain antifungal properties which inhibited growth of *R. solani* and *F. solani*. Moringa extract concentration levels influenced the antifungal efficacy of the extracts. Ejaz *et al.* (2017) found that extracts of *Moringa oleifera* Lam. plant parts (leaves, stem, fruit and seed) showed significant inhibition in the growth of *Macrophomina phaseolina* (Tassi) Goid, *Rhizoctonia solani* (Kühn) and *Fusarium oxysporum* (Schlecht) whereas stem extract and powder improved plant growth and showed maximum inhibition of root rot fungi on cow pea and mash bean plants crops under greenhouse condition. Debjani *et al.* (2017) found that leaves of clerodendrum (*Clerodendrum infortunatum* L.), polyalthia (*Polyalthia longifolia* Sonn.) and

rhizomes of ginger (*Zingiber officinale* Roscoe) were found most active against *Colletotrichum musae* and *Rhizoctonia solani* (Kühn) and showed significant inhibition of radial growth of all the test pathogen but the most effective plant extracts was clerodendrum extract, found to be most effective against rice sheath blight pathogen both in pot culture and field experiment.

## MATERIALS AND METHODS

### Preparation of plant extracts:

#### Aqueous plant extracts:

A quantity of the four different plants species namely (*Moringa*, *Lantana camara*, *Basil* and *Camphor*) chopped leaves each of plant species were washed several times with running tap water, then washed with sterile water and dried at room temperature ( $\pm 25\text{ }^{\circ}\text{C}$ ) for 15 days. Plant materials were ground to fine powders in a grinder, then 100g of each one were blended in 1L of soaked water for 48h. The macerated materials were squeezed through double cheesecloth sheets and then filtered through a Whitman No.1 filter paper. The fresh extracts were applied just after preparation at the rates of 20% from the original ones (Abdel-Monaim *et al.*, 2011).

#### Solvents of plant extracts:

In this experiment, ethanol organic solvents were used for preparation of plant extracts. The method was the same to the aqueous plant extracts except using organic solvent instead of distilled water. The extracted materials with each of the solvent were concentrated with rotary vacuum evaporator at  $50^{\circ}\text{C}$  for 6 hrs. The final concentration was prepared as those of water extracts 20% by adding water for organic solvent extract (Abdel-Monaim *et al.*, 2011).

### Effect of different concentrations of plant extracts on liner growth (*in vitro*):

The different concentrations of each extract were used as a control 0, 25, 50 and 100% of the ethanol and aqueous extracts were separately mixed with (PDA) medium before solidification (1:9 v/v, respectively), then poured in a sterile Petri-dish. Four plates for each concentration were inoculated at the center with discs (5mm in diameter) cut out from the periphery of 7 days old culture of each of the tested fungi. Liner growth of the tested pathogenic fungi was measured when each particular control filled to Petri-dish with fungal mycelial growth.

### Greenhouse experiments:

The same concentrations of a fore mentioned plant extracts in the first experiment were added to the soil infested with *Fusarium solani*, *F. oxysporum* and *Rhizoctonia solani* a set of six pots 35 cm (4 kg), with 7 seeds per pot, were used for each tested fungus at the rate of 100 ml/pot then seeds of canola were planted. The percentage of damping-off, root rot and wilt were recorded and statistically analyzed.

**Table (1):** English name, family and scientific name of the tested plants

English name	Family name	Scientific name
Moringa	Moringaceae	<i>Moringa oleifera</i>
<i>Lantana camara</i>	Verbenaceae	<i>Lantana camara</i>
Common basil	Lamiaceae	<i>Ocimum basilicum</i>
Camphor	Lauraceae	<i>Cinnamomum camphora</i>

## RESULTS

### ***In vitro* effect of aqueous and ethanol plant extracts on fungal growth of the tested pathogenic fungi:**

Multivariate analysis of variance (MANOVA) were performed to compare between different groups (control, aqueous, and ethanolic extracts), plant extracts (Moringa, common basil, *Lantana camara*, and camphor) and different fungi (*F. solani*, *F. oxysporum* and *R. solani*). Accordingly, plant extract, groups induced a highly significant difference in variable ( $p < 0.001^{***}$ ) (Table, 2). However, different fungi had no significant effect ( $p > 0.05$ ). Furthermore, interaction between plant extract and group and fungi and group revealed a significant difference.

Two-way analysis of variance was also performed to assess and compare the effect of different groups (control, aqueous, ethanol) and plant extracts within each fungal species. Accordingly, highly significant differences were found induced by plant extracts ( $p < 0.001^{***}$ ), groups ( $p < 0.001^{***}$ ), and interaction between plant ext. and group ( $p < 0.001^{***}$ ).

Data in (Table 2) indicate that the mycelial growth of the tested fungus was inhibited in response to the antifungal substance presented in the tested plant materials in all the tested extracts with different percentages. Moringa gives the highest effect on the linear growth of all causal organisms when recorded (5.0, 2.05) in aqueous and ethanol extract for *F. solani* respectively, (4.15 and 3.5) for *F. oxysporium* and (4.06 and 3.43) for *R. solani*. Followed by *Common basil* which scored (5.20, 4.36) in aqueous and ethanol extract for *F. solani* and (5.50, 5.0) for *F. oxysporium* and (5.60 and 5.0) for *R. solani*.

Meanwhile *Lantana camara* exhibited (7.32, 6.0) in aqueous and ethanol extract for *F. solani*, respectively and (7.10, 6.8) for *F. oxysporium* and (7.0, 6.01) for *R. solani*.

The lowest percentage was recorded with Camphor when recorded (8.5, 7.15) in aqueous and ethanol extract for *F. solani* respectively, (8.33, 8.00) for *F. oxysporium* and (8.2, 7.52) for *R. solani*.

### **Greenhouse experiment:**

#### **Effect of soil treatment with some plant extracts on percentage of damping-off, root rot and wilt diseases under greenhouse condition:**

The efficacy of aqueous and ethanol solvent extracts of (Moringa, Common basil, *Lantana camara* and Camphor) against damping off, root rot and wilt

diseases caused by (*F. solani*, *F. oxysporum* and *R. solani*) in Canola is shown in (Table 3). Soil treated with any the tested plant extracts recorded significant reduction of damping-off, root rot and wilt incidence caused by the three fungi compared with control. The extracts of Moringa recorded the highest reduction in both damping off and root rot diseases caused by any of the tested fungi followed by Common basil and *Lantana camara* extracts. Meanwhile Camphor recorded the lowest protection against damping-off, root rot and wilt incidence in this respect. On the other hand, the ethanol solvent extracts of all tested plants suppressed damping off, root rot and wilt incidence more than aqueous extracts.

## DISCUSSION

*In vitro* effect of aqueous and ethanol plant extracts on fungal growth of pathogenic fungus was studied *in vitro* indicated that the mycelial growth of the tested fungus was inhibited in response to the antifungal substance presented in the tested plant materials in all the tested extracts with different percentages. Moringa gives the highest effect on the linear growth of all causal organisms in aqueous and ethanol extract for *F. solani*, *F. oxysporum* and *R. solani* followed by Common basil and *Lantana camara* meanwhile the lowest percentage was recorded with Camphor.

Also, under greenhouse condition the efficacy of aqueous and ethanol solvent extracts of (Moringa, Common basil, *Lantana camara* and Camphor) against damping off, root rot and wilt diseases caused by (*F. solani*, *F. oxysporum* and *R. solani*) in canola was evaluated, soil treated with any the tested plant extracts recorded significant reduction of damping off, root rot and wilt incidence caused by the three fungi compared with control. The extracts of Moringa recorded the highest reduction in both damping off and root rot diseases caused by any of the tested fungi followed by Common basil and *Lantana camara* extracts. Meanwhile camphor recorded the lowest protection against damping off, root rot and wilt incidence in this respect. On the other hand, the ethanol solvent extracts of all tested plants suppressed damping off and root rot incidence more than aqueous extracts.

**Table (2):** Effect of different plant extracts on linear growth of pathogenic fungi

Fungi	Plant extract	(Mean ± SE)			2-way ANOVA		
		Control	Aqueous extract	Ethanol extract	Plant	group	Plant x group
<i>F. solani</i>	<b>Moringa</b>	9.00 ± 0.00 a	5.00 ± 0.58 ef	2.05 ± 0.58 g			
	<b>Common basil</b>	9.00 ± 0.00 a	5.20 ± 0.58 ef	4.36 ± 0.58 f	<0.001***	<0.001***	<0.001***
	<b>Lantana camara</b>	9.00 ± 0.00 a	7.32 ± 0.14 bc	6.00 ± 0.58 de			
	<b>Camphor</b>	9.00 ± 0.00 a	8.50 ± 0.29 ab	7.15 ± 0.58 cd			
<i>F. oxysporum</i>	<b>Moringa</b>	9.00 ± 0.00 a	4.15 ± 0.29 ef	3.50 ± 0.58 f			
	<b>Common basil</b>	9.00 ± 0.00 a	5.50 ± 0.58 d	5.00 ± 1.69 de	<0.001***	<0.001***	<0.001***
	<b>Lantana camara</b>	9.00 ± 0.00 a	7.10 ± 2.37 bc	6.80 ± 0.06 c			
	<b>Camphor</b>	9.00 ± 0.00 a	8.33 ± 0.19 a	8.00 ± 0.58 ab			
<i>R. solani</i>	<b>Moringa</b>	9.00 ± 0.00 a	4.06 ± 0.35 ef	3.43 ± 0.69 f			
	<b>Common basil</b>	9.00 ± 0.00 a	5.60 ± 0.06 d	5.00 ± 0.29 de	<0.001***	<0.001***	<0.001***
	<b>Lantana camara</b>	9.00 ± 0.00 a	7.00 ± 0.58 c	6.01 ± 0.01 D			
	<b>Camphor</b>	9.00 ± 0.00 a	8.20 ± 0.12 ab	7.52 ± 0.58 Bc			
Multivariate analysis of variance							
Source	df	F	Sig.				
Corrected Model	35	31.6	<0.001***				
Plant extract	3	102.2	<0.001***				
Fungi	2	1.2	0.315 ns				
Group	2	308.4	<0.001***				
Plant extract * Fungi	6	0.2	0.966 ns				
Plant extract * Group	6	26.3	<0.001***				
Fungi * Group	4	3.0	0.024*				
Plant extract*Fungi*Group	12	0.6	0.849 ns				

\*, \*\*, \*\*\*, significant at p&lt;0.05, 0.01, 0.001; ns non-significant at p&gt;0.05

**Table (3):** Effect of soil treatment with aqueous and ethanol extracts of *Moringa olifera*, *Ocimum basilicum*, *Cinnamomum camphora* and *Lantana camara* individually on the incidence of Canola damping-off, root rot and wilt diseases caused by *F. solani*, *F. oxysporum* and *R. solani* under greenhouse condition

Source of plant extracts	Solvent type	<i>F. solani</i>		<i>F. oxysporium</i>		<i>R. solani</i>	
		% Damping off	% Root rot	% Damping Off	% Wilt	% Damping Off	% Root rot
Moringa	Aqueous extract	15.0	18.25	15.0	14.25	15.0	10.00
	Ethanol extract	10.0	14.25	10.0	12.25	10.0	7.25
	Mean	12.5	16.25	12.5	13.25	12.5	8.62
Common basil	Aqueous extract	20.0	23.75	15.25	14.0	25.0	15.75
	Ethanol extract	19.0	18.75	10.25	12.25	15.0	11.25
	Mean	20.0	21.25	12.0	13.12	20.0	13.45
Lantana camara	Aqueous extract	23.0	25.0	18.25	17.0	27.0	17.25
	Ethanol extract	20.0	20.0	12.25	12.25	17.0	12.25
	Mean	21.5	22.5	15.25	14.6	22.0	14.75
Camphor	Aqueous extract	25.0	27.25	20.0	17.0	30.0	17.75
	Aqueous extract	20.0	24.25	15.0	13.75	20.0	12.25
	Mean	22.5	25.75	17.5	15.25	25	15.0
Control		45.0	40.25	60.0	45.0	60.0	30.33
<b>L.S.D at 0.05</b>							
Plant extracts (A)		2.20	1.18	2.93	1.78	3.02	1.82
Solvents types (B)		0.84	0.53	1.08	0.60	1.04	0.6
Interaction (A*B)		3.02	1.75	4.09	2.43	4.2	2.0

This may be due to the solvent that has potential to extract the different constituents that having antimicrobial activity. The inhibitory effect of the tested extracts might be due to natural bioactive materials present in these extracts (Abdel-Monaim *et al.*, 2011; Atta *et al.*, 2013). Some authors reported that leaves of *M. olifera* contain phytochemicals like tannins, sterols, saponins, terpenoids, phenolics alkaloids and flavanoids like quercetin, kaemfericetin and glycoside compounds Goss *et al.* (2017) who proved that Moringa leaf and seed extracts contain antifungal properties which inhibited growth of *R. solani* and *F. solani*. Moringa extract concentration levels influenced the antifungal efficacy of the extracts. Moreover, ethanolic extract of *M. olifera* leaves showed antifungal activity against a number of dermatophytes (Chuang *et al.*, 2007). The efficacy of 13 plant extracts viz-leaf extracts of *Azadirachta indica*, *Catharanthus roseus*, *Lantana camara*, *Ocimum sanctum* *Ricinus communis*, *Saraca indica* and *Thuja occidentalis*, latex yielding plants *Calotropis procera*, *Nerium indicum*, *Datura*, *Ficus religiosa* and bulbs of *Allium cepa* and *Allium sativum* were tried to control root rot pathogen *in vitro*. They were evaluated for their antifungal activity over *Rhizoctonia solani*. All the plant extracts

showed significant reduction in the growth of the pathogen compared with control Nupur and Rashmi (2015). *Datura* extracts was the most effective against *Rhizoctonia solani*, *Fusarium solani* and *Sclerotium rolfsii*, followed by Spairment while Neem and Common basil had moderate effect EL-Korashy (1997).

Also other investigators found an antifungal activity of some natural plant products suppressed plant pathogens with an increase of oxidative enzymes in plants that can play important role in the resistance to infection with diseases and consequently increasing growth parameters and seed yield (Abdel-Monaim *et al.*, 2011).

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## تأثير بعض المستخلصات النباتية كوسيلة آمنة لمكافحة موت البادرات وأعفان جذور وذبول الكانولا

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يعتبر مرض موت البادرات، أعفان الجذور والذبول من أخطر الأمراض التي تسبب خسائر كبيرة في إنتاجية المحصول وجودته. في هذه الدراسة تم عزل، رايزوكتونيا سولاني وفيوزاريوم سولاني وفيوزاريوم اوكسيسبوريم من جذور نباتات الكانولا المصابة بأعفان الجذور والذبول. كما أظهرت المستخلصات النباتية لكل من لانتانا كامار ومورينجا وأوليفيرا وبازل وكافور تأثيرات مختلفة على تثبيط النمو الميسيليومي لجميع الفطريات المختبرة مقارنة بالكنترول وكان مستخلص المورينجا الأعلى كفاءة في التثبيط. تحت ظروف الصوبة، حيث تبين أن جميع المستخلصات أدت إلى انخفاض معنوي في حدوث الإصابة بأمراض موت البادرات وأعفان الجذور وكان مستخلص المورينجا الأعلى كفاءة بينما كان مستخلص الكافور أقل المستخلصات كفاءة وأعطى مستخلص الكحول الإيثيلي نتائج أفضل في مقاومه الفطريات عن المستخلص المائي.