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Efficacy and Safety of Some Plant Extracts against Tomato Early Blight Disease Caused by *Alternaria solani*

[1]A.S. Derbalah, [2]M.S. El-Mahrouk and [3]A.B. El-Sayed
[1]Department of Pesticides,
[2]Department of Horticulture, Faculty of Agriculture, Kafr-El-Shiekh University, 33516, Egypt
[3]Institute of Plant Pathology Research, Agriculture Research Center, Egypt

Abstract: In an attempt to establish new control practices with low mammalian toxicity and low persistence in the environment against plant pathogens, crude extracts of seven plant species (*Cassia senna*, *Caesalpinia gilliesii*, *Thespesia populnea* var. *acutiloba*, *Chrysanthemum frutescens*, *Euonymus japonicus, Bauhinia purpurea* and *Cassia fistula*) were evaluated against *Alternaria solani* in tomato under laboratory and greenhouse conditions. Furthermore, GC-MS analysis was carried to identify the biologically active components of the most effective extract against *A. solani*. Moreover, the safety of the most effective extract was evaluated with respect to histological changes in treated rats' organs. The results showed that, *B. purpurea* was most effective plant extract against early blight pathogen under laboratory and greenhouse conditions. The GC-MS analysis for the most effective plant extract showed the presence of different bioactive chemical components than known by its antifungal activity. The most effective plant extract showed low toxicity on rats relative to control. The results revealed that, the using of plant extracts can be regarded as effective and safe control of *A. solani* in tomato.

Key words: Extract, control, pathogen, fungicides, tomato

INTRODUCTION

More than 800 million people in developing countries do not have adequate food supplies and at least 10% of food is lost due to plant diseases (Strange and Scott, 2005). Plant diseases are caused by pathogens such as fungi, bacteria, nematodes and viruses. Compared to other plant parasites, fungi cause the greatest impact with regard to diseases and crop production losses either foliage or post harvest losses of fruits and vegetables through the decay resulted from fungal plant pathogens.

Tomato (*Lycopersicon esculentum* Mill.) is an important vegetable crop grown throughout the world. Tomato early blight disease caused by *Alternaria solani* become the most destructive in all over the world and yield losses up to 80% (Singh, 1985; Mathur and Shekhawat, 1986; Chandravanshi *et al*., 1994). The control of tomato early blight disease has been almost exclusively based on the application of chemical pesticides. Several effective pesticides have been recommended against this pathogen but they not considered a long-term solution, due to concerns of expense, exposure risks and the hazards of its residues. Moreover, the development of resistance of pathogenic fungi towards synthetic pesticides is a great problem that can affect significantly the efficacy of chemical fungicides. Thus to find safe, efficacious and environmentally friendly fungicides considered as source of major concern (Mdee *et al*., 2009).

Presently, the search for natural products with novel uses, particularly related to pest management is very important task. The use of plant extracts has been shown to be eco-friendly and effective against many plant pathogens (Khallil, 2001; Hawamdeh and Ahmad, 2001; Saadabi, 2006; Gachomo and Kotchoni, 2008; Thobunluepop, 2009; Latha *et al*., 2009; Moslem and El-Kholie, 2009; Duru and Onyedineke, 2010). Most of these substances were evaluated in order to find a safe alternative control methods to the human and the environment. Therefore, research should focus not only on the efficacy of botanical extracts against the target pests but also their safeties on human health in demand. Although, the assessment of enzymes activity in the blood is generally a sensitive measure of compounds toxicity than histopathological changes that can be assessed within a shorter time. The tissue alterations considered a confirmatory and supporting diagnostic role in the case of certain abnormalities in blood sampling (Cornelius *et al*., 1959). Most of the selected extracts in

Corresponding Author: Aly Soliman Derbalah, Department of Pesticides, Faculty of Agriculture, Kafr El-Sheikh University, 33516, Kafr El-Sheikh, Egypt Tel: 204732232868 Fax: 20473232032

this study were known by their natural origin and safety to human health (Park *et al*., 2005; Panda and Kar, 1999). Moreover, no evidence of teratogenic or genotoxic activity has been detected by using of plant extracts to control pests (Mengs *et al*., 2004; Mitchell *et al*., 2006). This is besides the fact these plants are available in high amount in Egypt.

Therefore, the present work was designed to investigate the efficacy of certain plant extracts against the early blight pathogen, caused by *A. solani*, under laboratory and greenhouse conditions. This study has also to identify of the biologically active components of the most effective plant extract against the tested plant pathogen. Finally, it has been done to evaluate the toxicity of the most effective plant extract on rats with respect to histology changes in treated rats' organ relative to control.

MATERIALS AND METHODS

Plants and preparation of crude extracts: The leaves of seven medicinal plant species (*Cassia senna*, *Caesalpinia gilliesii*, *Thespesia populnea* var. *acutiloba*, *Chrysanthemum frutescens*, *Euonymus japonicus, Bauhinia purpurea* and *Cassia fistula*) were collected from a local nursery at Kafr El-Sheikh, Monofia, Gharbia and Alexandria Governorates, Egypt. *C. senna* (Alexandrian Senna) belonging to the family Fabaceae, is native to tropical Africa and cultivated in Egypt and Sudan. *C. gilliesii* (bird of paradise) belonging to the family Fabaceae, is native to tropical America, mainly Argentina and Uruguay. *T. populnea* var. *acutiloba* (Portia Tree) belonging to the family Malvaceae, is native to South Africa. *C. frutescens* (marguerite daisy) belonging to the family Asteraceae, is native to the Canary Islands. *E. japonicus* (Japanese Spindle) belonging to the family Celastraceae, is native to Japan, Korea and China. *B. purpurea* (Purple camel's foot) belonging to the family Fabaceae, is native to South China. *C. fistula* (Cassias) belonging to the family Fabaceae, is native to southern Asia. The different leave samples were oven dried for 24 h at 70°C and then, finely ground into a powder using a blender. Each sample (25 g) was extracted twice with 300 mL of methanol at room temperature for 2 days. The extracts were filtered through Whatman filter paper. The combined filtrate was concentrated to dryness by rotary evaporation at 40°C.

Pathogen and plant cultivar source: The *A. solani* isolated from tomato plant was obtained as culture slant from the Department of Mycology and Plant Disease Survey, Plant Pathology Research Institute, Giza Egypt. Tomato, (*Lycopersicon esculentum*) plant, cultivar Super Strain, was obtained from Legumes Department, Gemmiza Agriculture Research Station. Cultures were grown on potato dextrose agar in 9 cm diameter Petri dishes for 14 days and incubated at 28±1°C. The resultant fungal growth was providing the amount of water used amounts of sterilized distilled water for 2 min. The suspension was filtered through two layers of sterilized cheesecloth. The spore suspension was counted using a hemocytometer under the microscope and was adjusted to 10^6 spores mL^{-1} according to the methods described by Shahin and Shepared (1979).

The tested fungicide: The tested fungicide used in this study was metalaxyl with a trade name of vicomil 50% WP used at field rate of five 370 g ha^{-1} and produced by Kafr-El-Zayat Company for chemicals and pesticides, Egypt. This fungicide recommended for control early blight disease in vegetables crops in Egypt.

Screening of plant extracts efficacy against *A. solani* under laboratory conditions: The seven extracts and metalaxyl were tested for their efficacy against *A. solani* in a completely randomized design. The efficacy of the tested plant extracts and fungicide was determined as percent of inhibition in the growth of selected fungus relative to the control treatment. Four concentrations for each plant extract (50, 100, 150 and 200 ppm) and four concentrations for the fungicide (1, 10, 25 and 50 ppm) were used. The required concentrations for plant extracts and fungicide were obtained by adding the appropriate amount of stock solution used to 60 mL portions of auto-calved PDA cooled to about 45°C. Four Petri dishes, 9 cm in diameter, were used as a replicate for each concentration of each treatment, including control. Control treatment was carried out without adding fungicide or plant extracts. Each dish was inoculated in the center with a disk (5 mm diameter) bearing the mycelium growth from *A. solani* culture (5 days old culture). The dishes were sealed with parafilm to avoid the evaporation of volatile compounds. The Dishes were incubated at 28°C until the full growth (mycelium reaching the edge of the plate) of the control treatment. The inhibition percentage of radial growth of *A. solani* was calculated using the formula suggested by Vincent (1947). Each the experiment (all concentrations for each treatment) was repeated three times. The inhibition percentage was calculated as shown in Eq. 1:

$$\%I = \frac{A - B}{A} \times 100 \quad (1)$$

where, A is the radial growth of the tested fungus in control, B is the radial growth of the tested fungus in treatment and %I is percentage of radial growth inhibition.

Efficacy of the tested plant extracts against *A. solani* under greenhouse conditions: These experiments were carried under greenhouse conditions. Seeds of tomato (*L. esculentum*), cultivar Super Strain were planted in 17 cm diameter plastic pots filled with 2 kg/each of un-sterilized-loamy-clay soil. The plants were irrigated normally and the treatments were applied on the plants after 4 weeks from planting. The highest concentrations of tested plant extracts (150 and 200 ppm) were applied as foliar treatment on tomato early blight grown under greenhouse conditions. Metalaxyl was used as standard fungicide against *A. solani* at recommended dose level. Tomato plants in control were sprayed with water only at the same intervals used in plant extracts application. The tested plant extracts were applied twice, once at 15 days prior to inoculation with the pathogen and once 7 days before inoculation. The inoculation of tomato plants with *A. solani* spore suspension at concentration level of 10^6 spore mL^{-1} was carried out by sprayed it on tomato seedlings 10 days after transplanting using an atomizer. Inoculated seedlings were covered with black polyethylene bags for 48 h and kept in the green house at 30-32°C. Diseased plants were assessed weekly starting with the first symptom appearance till the end of the growing period. Twenty five leaves for every treatment were used to record the disease parameters according to the method of Awad (1980). Disease severity was assessed 8 days after inoculation according to the scale of Chirst (1991) and the efficacy of each treatment was calculated using Eq. 1:

$$\%\text{Efficacy} = \frac{\text{DSC} - \text{DST}}{\text{DSC}} \times 100 \quad (2)$$

Where:
DSC = Disease severity under control
DST = Disease severity under treatment

Chemical composition of the most effective plant extract: GC/MS analysis was carried to identify the components of the most effective plant extract (*B. purpurea*) according to the method described by Duarte-Almeida *et al.* (2004).

Toxicity assessments: The used adults Wistar male rats (*Rattus norvegicus*) with 8 weeks old and 80-100 g in weight were obtained from Faculty of Medicine, Tanta University. Wister rats were housed in wire cages under standard conditions with free access to drinking water and food. The rats were kept in temperature-controlled room with 14 h light and 10 h dark cycles. The rats were given a standard diet as described by Romestaing *et al.* (2007). Before treatment, rats were left two weeks during feeding for adaptation. The animals were randomly divided into two groups each comprising of three animals. The first group was for the treatment with the most effective plant extract (21 days) and the second group was for control. The most effective plant extract (*B. purpurea*) against the tested fungus were administered one time to rats orally at concentration level of 500 mg kg^{-1} body weight. Rats in control treatment were orally administrated with equal amount of almond oil. After 21 days of treatment, the rats were sacrificed under anesthesia. Then, specimens from kidney and lung were taken from each treatment and kept in neutral buffered formalin 10% for histopathological tests. The histopathology test was carried out at Histopathology Laboratory, Department of Histopathology, Faculty of Veterinary Medicine, Kafr El-Sheikh University according to the method described by Bancroft *et al.* (1996).

Statistical analysis: Data were subjected to the analysis of variance test and Newman-Keuls's multiple range test using a computer program SAS (Version 6.12, SAS Institute Inc., Cary, USA). The level of significance was 0.05.

RESULTS

Effect of the tested plant extracts on radial growth of *A. solani*: Seven plant species, belonging to the various families were selected and evaluated for their antifungal activity against *A. solani*, the causal of early blight disease in tomato. All the tested plant extracts at different concentration levels inhibited the radial growth of *A. solani*, relative to control. The leaf extract of *T. populnea var. acutiloba* was the most effective one against *A. solani* with the inhibition percentage of 79.4%, followed by *C. frutescens, C. gilliesii, C. senna, E. japonicus, B. purpurea* and *C. fistula* with inhibition percentages of 73.3%, 71.1, 67, 59.4, 56.4 and 54.2%, respectively (Table 1). However, the fungicide metalaxyl as a recommended compound against *A. solani* was still the most effective treatment compared to all tested botanical extracts. The efficiency of the tested botanical extracts was concentration dependent since it's toxicity against *A. solani* increased with the increasing of their concentrations level.

Efficacy of plant extracts on tomato early blight pathogen under greenhouse conditions: The protective action of the tested plant extracts relative to the recommended fungicide metalaxyl against *A. solani* in Tomato plant that evaluated under greenhouse conditions is shown in Table 2. The results showed that metalaxyl was the most effective treatment against *A. solani* followed by *C. gilliesi, T. populnes* var. *acertiloba, C. frutesscens, C. fistula, C. senna, B. purpurea* and *E. japnicus*,

Table 1: Efficacy of the tested plant extracts and metalaxyl against early blight disease pathogen (*A. solani*) under laboratory conditions

Treatments	Concentrations	Inhibition percentages
C. senna	50	27.7a
	100	34.5d
	150	39.8i
	200	59.4j
C. gilliesii	50	32.3v
	100	51.5fg
	150	59.0m
	200	73.3pq
T. populnea var. *acutiloba*	50	24.8igh
	100	31.6r
	150	34.2c
	200	56.4f
C. frutescens	50	32.7q
	100	39.5fgh
	150	45.5j
	200	71.1L
E. japonicus	50	25.5q
	100	31.0u
	150	33.9c
	200	67.0fe
B. purpurea	50	44.7t
	100	57.5L
	150	68.8po
	200	79.4s
C. fistula	50	21.7ih
	100	29.7o
	150	42.9b
	200	54.2e
Metalaxyl	1	33.5c
	10	68.7n
	20	86.2k
	50	98.0x
Control	0	0.00y

Lower case letters in this column indicate separation of means according to the Student Newman Keuls multiple range test ($p<0.05$)

Table 2: Efficacy of the tested plant extracts and metalaxyl against early blight disease pathogen (*A. solani*) under greenhouse conditions

Treatments	Disease severity	%Efficacy	Disease severity	Efficacy
C. senna	13.0d	47.60	9.02cde	66.00
C. gilliesii	10.8e	56.47	9.46de	64.31
T. populnea var. *acutiloba*	17.6b	29.03	9.99cd	62.31
C. frutescens	11.25e	54.66	9.56d	63.97
E. japonicus	15.4e	28.00	10.85bc	59.04
B. purpurea	9.7e	61.00	8.07e	69.55
C. fistula	12.65d	49.00	9.63cde	63.67
Metalaxyl	7.57f	69.50	5.24g	80.20
Control	24.81a	0.00	26.50a	0.00

Lower case letters in this column indicate separation of means according to the Student Newman Keuls multiple range test ($p<0.05$)

respectively either at concentration level of 150 or 200 ppm. The results also implied that the efficacy of the plant extracts was higher at a concentration level of 200 ppm than that of 150 ppm (efficacy was concentration dependent). There was no phytotoxicity of the tested plant extracts on tomato was observed Moreover, one of the most important notices that, there is no phytotoxicity of the tested plant extracts on tomato was recorded.

Composition of the most effective botanical extract against *A. solani*: The identified compounds from the most effective botanical extract (*B. purpurea*) against *A. solani* are given in Table 3. Eighteen compounds were identified from *B. purpurea* plant extract as shown in Table 3. The identified compounds are belonging to aldehydes, esters, alcohols and fatty acids.

Table 3: The main constituents of *B. purpurea* extract identified by GC-MS analysis

Identified compounds	Retention time (min)	% Area
Eugenol	8.37	5.80
Alpha humulene	9.15	2.56
Myristicin	9.71	0.88
Elemicin	9.90	0.69
Tetradecanoic acid methyl ester	11.17	0.66
Tetradecanoic acid	11.61	3.52
Mone inositol	11.97	8.73
2-methyl -1- thia cyclopentene	12.05	5.09
Acrylic acid	12.19	6.60
4,6 Di-o-methyl-alpha d galactose	12.43	4.15
Methylthiolane	12.57	5.47
Pivalion	12.61	5.20
Pentadecanoic-4-methylethyl ester	13.07	2.53
n- hexadecanoic acid	13.60	7.72
9,12,15 octadecatrienoic methyl ester	15.13	2.56
Phytol	15.28	12.8
Oleic acid	15.70	3.18
9,12, 15 octadecanoic acid	15.78	2.93

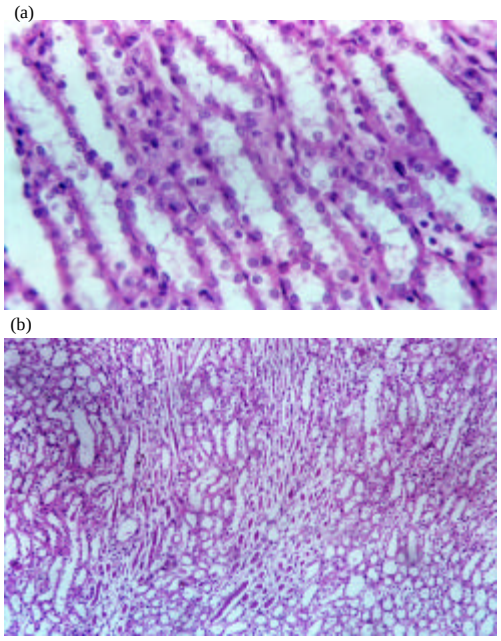

Fig. 1(a-b): Sections in kidney of rats treated with *B. purpurea* (b) at dose level of 500 mg kg^{-1} after 21 days of treatment relative to control (a)

Toxicity evaluation

The histopathological changes in the kidney: The normal structure of kidney tissue was shown in Fig. 1a. For the rats treated with *B. purpurea* extract at dose level of

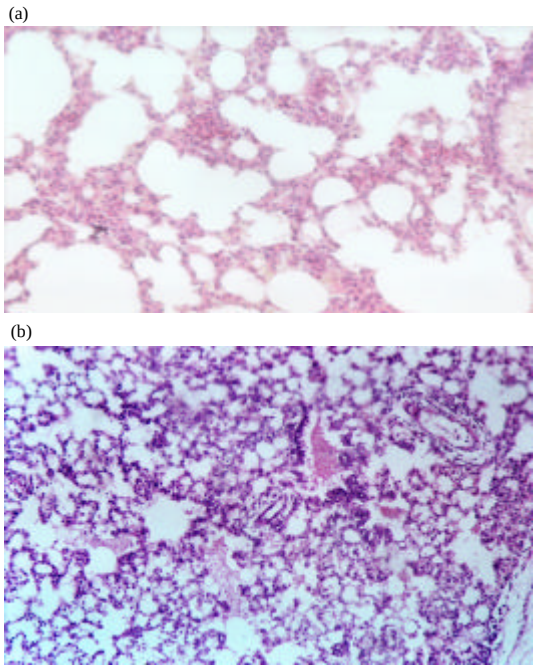

Fig. 2(a-b): Sections in lung of rats treated with *B. purpurea* (b) at dose level of 500 mg kg^{-1} after 21 days of treatment relative to control (a)

500 mg kg^{-1}, the kidney tissue was the lung tissue is normal as control except for some collecting tubules (Fig. 1b).

The histopathological changes in the lung: The normal structure of lung tissue was shown in Fig. 2a. For the rats treated with *B. purpurea* extract at dose level of 500 mg kg^{-1}, the tissue was normal as control with some lymphocytes infiltration and the blood vessels were observed to be engorged with blood (Fig. 2b). However, these changes still not significant relative to the normal tissue.

DISCUSSION

In the present study, the tested plant extracts showed antifungal activity against early blight pathogen in tomato. The efficacy of different plant extracts belonging to different species other than the tested botanical extracts against the *A. solani* either under laboratory or greenhouse conditions have been reported (Bergaoui *et al*., 2007; Tegegne *et al*., 2008; Chutia *et al*., 2009; Latha *et al*., 2009; Zaker and Mosallanejad, 2010). However, for the tested plant extracts especially the effective ones against early blight pathogen *A. solani*, this is considered the first report.

It was observed that, among the identified compounds from *B. purpurea* extract, some compounds such as, Alpha humulene , eugenol, tetradecanoic acid, oleic acid, Phytol, 9, 12, 15 octadecanoic acid and n-hexadecanoic acid were detected with high percentages relative to other detected compounds. The antifungal activity of *B. purpurea* extract against *A. solani* pathogen may be due to the presence of the previous fatty acids and its derivatives (Hammer *et al*., 2003; Bergaoui *et al*., 2007; Chutia *et al*., 2009; Ahmadi *et al*., 2010).

Although, the antifungal activity of tested plant extracts is mainly attributed to its major compounds but the synergistic or antagonistic effect of one compound in minor percentage in the mixture has to be considered (Ragasa *et al*., 2002). Therefore, each component of the plant extract has its own contribution on biological activity of the extract.

The essential oils in the tested plant extracts as antimicrobial agents considered at low risk for resistance development by pathogenic microorganisms. It is believed that it is difficult for the pathogens to develop resistance to such a mixture of components with, apparently, different mechanisms of antimicrobial activity (Liu *et al*., 2008).

The botanical extracts as pest control agents present two main characters: the first is their safety to the people and the environment and the second is the less resistance development against it by the tested pathogen. Regarding to the safety, the toxicity evaluation of the most effective plant extract revealed that, there were some slight variations occurred sporadically in treated rats relative to control with the respect to enzymes markers and histopathology of treated organs. Moreover, the observed changes in the tissues were mostly uncorrelated with the dosages which mean the safety of the tested plant extract on human health. With the referring to resistance development, it is believed that, it is difficult for the insect to develop resistance to such a mixture of bioactive components with, apparently, different mechanisms of fungicidal activity (Liu *et al*., 2008).

CONCLUSIONS

The fungicidal activity of the extracts against *A. solani* indicates the potential of some plant species as a natural source of fungicidal material. Antifungal activity was confirmed in all the tested plant species, although the results showed that different plant extracts varied in their effectiveness in inhibiting the mycelia growth of different pathogens tested. *In vivo* results under greenhouse confirmed and could be a viable option for controlling *A. solani* because leaves are available in all seasons and

it is a world wide invasive species. The ability of using botanical products as alternative of chemical control of plant pathogens is possible. This approach can contribute in reducing the amount applied of fungicides and subsequently minimize its hazards to the environment and human health. Work in this regards should continuing on other invasive species on isolating antifungal compounds and on field trials with promising extracts or compounds. Also, further research is needed in order to obtain information regarding the practical effectiveness of essential oils to protect the plants or the plant products without toxic effects.

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