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Antiviral Properties of *Artemisia absinthium* and *Allium cepa* Extracts against Potato Virus Y (PVY)

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The present study sought to investigate the potential use of plant derived products in the treatment and prevention of plant diseases with the aim of reducing the yield loss one of the most important crops: Potato. Plantextracts are prominent and are cultivated worldwide and/or are used commercially. The present study evaluated, both *in vitro* and *in vivo*, the antiviral properties of two plants extracts (*Artemisia Absinthium* and *Allium cepa*). *Chenopodium amaranticolor* was used as a local lesion host. The inhibition percentages of PVY by both plant extracts with varying dilutions were recorded over a period of 5 days. A higher percentage of viral inhibition was observed *in vitro* with the aqueous extract of *Artemisia Absinthium*(72.73%)than with *Allium cepa* (42.42%).PVY inhibition was more effective pre-inoculation than post-inoculation, and the higher percentage of inhibition was observed as the period of treatment lengthened with both plant extracts. For the pre-inoculation treatment, the highest percentage of inhibition with the aqueous extract of *Artemisia Absinthium* was 54.54% observed after 5 days, in contrast with 36.36% with the aqueous extract of *Allium cepa* during the same period. Much lower percentages of inhibition were observed with the post-inoculation treatment (45.45% and 24.24% respectively). The study concluded that *Artemisia Absinthium* has a stronger inhibitory effect against PVY infectivity than *Allium cepa*.

Key words: *Artemisia Absinthium, Allium cepa,* Potato Virus Y, PVY, Inhibition.

Potato Virus Y (PVY) is a member of the *Potyviridae* family of viruses and is widespread globally, but with a narrow host range, affecting various solanaceous crops such as tomato, pepper, tobacco, and potato. This family of viruses is renowned for causing significant losses in plants with economic importance, including but not limited to, cereals, vegetable, fruit, sugarcane, and oilseed. The PVY flexuous particles have a model length of 740nm and an 11nm width[1]. The genome is composed of 9704 nucleotides. PVY is a yield-limiting virus where infection may lead to severe damage in potato plants (*Solanum tuberosum* L.) leading to high yield loss, of between 50-80%, when levels surpass established tolerances. The virus may also induce post-harvest losses due to tuber necrosis and reduced storage quality. PVY can be transmitted mechanically or in a non-persistent manner by a range of Aphid species, which can acquire the virus from an infected plant in less than 60 seconds and transmit it to a healthy one in also less than 60 seconds. Aphids may retain the virus for more than 24 hours if feeding does not take place.

Medicinal plants are prominent and are cultivated worldwide and/or are used commercially. They are said to represent a vital health and economic component of biodiversity. The Arabian Peninsula is the birth place of herbal drugs[2] and the use of folk medicine has existed in the region since time immemorial. The Kingdom of Saudi Arabia has a wide range of flora that comprises a

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large number of medicinal herbs, shrubs and trees[3]. Saudi Arabia is estimated to have a great medicinal species diversity that is expected to be over 1200 (above 50%) out of its 2250 species.

Plant extracts and products have been observed to be effective against a large number of pathogens[4]. Similarly, viral pathogens and fungal, bacterial and viral pathogens have been mainly controlled through the use of plant seed oils[5, 6, 7, 8]. Resistance against plant viruses had also been introduced by several studies through chemicals amongst which is salicylic acid which is a natural agent used for TMV control[9].

Many approaches have been used for the control of plant viruses such as the use of chemicals, breeding, cross-protection and transgenic plants. Despite this, once a plant becomes infected, there are no control measures which can completely inhibit plant viruses. The discovery of plant extracts possessing antiphytoviral activities led to the debate on the selection of the most suitable and effective natural products with antiviral activities that can inhibit plant viruses. Numerous crude extracts from plants were screened to determine their antiviral activity against plant viruses[10, 11]. Many virus-inhibiting constituents were isolated from higher plants including proteins[12,13], flavonoids[14, 15]. A large number of plant products and extracts have also been found to have an effective role against a wide spectrum of pathogens[16,17,18].

The present study aimed to highlight the potential use of plant derived products as antiviral agents. *Artemisia* is a large and diverse genus of plants consisting of nearly 400 species that belong to the *Asteraceae* daisy family. The genus is well known for powerful chemical constituents in their essential oils. The *Artemisia* species grow in the temperate climates of both hemispheres, commonly in dry or semiarid habitats. White hairs cover the leaves of many species. Most *Artemisia* species possess strong aromas and bitter tastes as a consequence of the terpenoids and sesquiterpene lactones present in the plant. Plants in the *Artemisia* genus grow best in free-draining sandy soil that is unfertilised and under full sun[19].

On a similar note, vegetables belonging to the *Allium* family have been used extensively in traditional medicine in the treatment of a wide range of diseases. Onion (*Allium cepa L.*) is infamous for its pungency, flavour, and medicinal attributes. The medicinal effect of onion bulbs has been widely investigated[20,21]. Onions have been observed to be rich in flavonols and organosulphur compounds[22,23,24]. In the effective control of plant viral diseases, effective viricides are lacking. Safer botanical extracts are gaining importance in recent years in the field of crop protection against pests and diseases[25].

MATERIALS AND METHODS

Preparation of Aqueous Plant Extracts

Aqueous extracts were obtained by grinding 100g of onion bulb (*Allium cepa*) and fresh leaf of *Artemisia absinthium*, separately, each with 100 mL of phosphate buffer (0.01M, pH 7.2) (1:1 w/v) using pestle and mortar. The extract was filtered through double-layered cheese cloth. The filtrate was sonicated and centrifuged at the rate of 10,000 rpm for 10 min. The supernatant was used for further studies. The supernatant was collected and stored at -15^{o}C[26]. Extracts were diluted by distilled water to 10^{-1}, 10^{-2} and 10^{-3} before use.

Virus source and preparation of standard virus inoculum

The PVY isolate was obtained from Ain–Shams University, Faculty of Agriculture, Cairo, Egypt, from*Nicotianatabacum L.* leaves showing veinal necrosis and mosaic symptoms. The leaves were homogenised in Phosphate buffer (1:3 w/v) at a pH 7.7 in a pre-chilled mortar. Following homogenisation, the extract was filtered through muslin cloth and the supernatant (juice) was used as the source of the inoculum.

Mechanical inoculation and viral propagation

The juice produced in step 1 above was used to inoculate *Chenopodium amaranticolor* after three weeks of planting. The leaves were lightly dusted with Carborundum, and then inoculated with the PVY-containing juice produced in step 1. Plants were placed back into the greenhouse after the inoculation. Symptoms for infection with PVY became apparent 2 weeks post-inoculation. Symptomatic plants were used as source for further experimentation.

Virus purification

The virus was purified from local cultivars to be tested for further *in vitro* experiments. As described above, plants were propagated and

infected with the virus. The purification procedure was completed[27,28] but with minor modifications. Plant leaves with an original leaf weight ranging from 11.25g and 15.20g and showing PVY symptoms were selected. Fresh leaf tissue was pulverised in liquid nitrogen in a large mortar placed over ice. The leaves were thoroughly homogenised in the phosphate buffer 1M (K_2HPO_4/ KH_2PO_4) pH 7.0. The homogenate was filtered through four layers of muslin cloth until a fine homogenate was produced. The virus was purified using chloroform (1:10) with little shaking. This was thencentrifuged at low speed for 20 minutes at 3000rpm. The top layer was transferred to a new centrifuge tube and was ultra-centrifuged at a high speed of 40000rpm for a period of 2 hours. The whole top layer was then removed and the pellet was taken. The pellet was gently re-suspended in 100µl of phosphate buffer 1M (K2HPO4/ KH2PO4) at pH 7. The virus preparation was negatively stained using uranyl acetate 2% and was examined using Transmission Electron Microscopy (TEM).

Investigating the effect of plant extracts on PVY infectivity *in vitro*

To test the effect *Artemisia Absinthium* and *Allium cepa* plant extracts on PVY infectivity at different time intervals (5 days) in vitro, 2 ml of virus containing sap were mixed with 2 ml of each plant extract and allowed to stand for one week. The mixtures were then inoculated into 3-weeks old *Chenopodium amaranticolor* at days one, two, three, four and five. For the control, distilled water was used. The developed local lesions were counted and the percentage of inhibition was calculated using the formula[21,29].

% Inhibition = (control -treatment) x100/ control

Inoculated plants were placed in an insect-proof greenhouse until symptoms developed. Each experiment was repeated three times to account for any possible discrepancy in susceptibility amongst host plants.

Investigating the effect of plant extracts on PVY infectivity *in vivo*

Pre-inoculation treatment

To test the effect of *Artemisia Absinthium* and *Allium cepa* plant extracts on PVY infectivity *in vivo,* 2 ml of each plant extract were rubbed on the leaves *Chenopodium amaranticolor* plants for 30 minutes. After one hour, each plant was inoculated with 2ml PVY infected sap. Again, for the control, distilled water was used.

Post-inoculation treatment

The same steps as in the pre-inoculation treatment was adopted, however, treatment with plant extracts was applied one hour following the inoculation of the *Chenopodium amaranticolor* plants with infected sap. Again, inoculated plants were placed back into the insect-proof greenhouse until symptoms developed. Each experiment was repeated three times to account for any possible discrepancy in susceptibility amongst host plants.

RESULTS

Effect of the aqueous extracts and dilutions of *Artemisia absinthium* and *Allium cepa* on PVY infectivity *in vitro*

The results in tables 1 and 2 demonstrate that the aqueous extracts of both *Artemisia absinthium* and that of *Allium cepa* possess an inhibitory effect against PVY infectivity *in vitro.* The highest percentage of inhibition was observed with the aqueous extract of each plant. As the dilutions increased from 10^{-1} to 10^{-3} the inhibitory effect decreased. With the aqueous extract of *Artemisia absinthium*, the highest percentage of inhibition (72.73%) against PVY infectivity was observed after 5 days. On the other hand, the highest percentage of inhibition against PVY infectivity observed with the aqueous extract of *Allium cepa* was 42.42% after 5 days. Figures 1 and 2 demonstrate that the aqueous extract and dilutions of *Artemisia absinthium* were significantly more inhibitory than that of *Allium cepa* with the highest dilution of *Artemisa absinthium* 10^{-3} achieving a PVY percentage inhibition of 45.45% after 5 days, which remains higher than the PVY percentage inhibition observed with the aqueous extract of *Allium cepa* after the same period (42.42%). *In vitro* tests show that *Artemisa absinthium* was more effective at reducing the number of local lesions produced by PVY infection on *Chenopodium amaranticolor* than *Allium cepa*.

Effect of the aqueous extracts and dilutions of *Artemisia absinthium* and *Allium cepa* on PVY infectivity *in vivo*

The results obtained in tables 3 and 4; respectively demonstrate that that the aqueous extracts of both *Artemisia absinthium* and that of

Table 1. Effect of the aqueous extract and dilutions of *Artemisia absinthium* on the number of local lesions developed by PYV infection on *Chenopodiumamaranticolor in vitro*

Time (Days)	Mean Number of Local Lesions								
	Control	Aqueous Extract	% Inhibition	Dilutions			% Inhibition		
				10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
1	39	23	41.03	25	28	35	35.90	28.21	10.26
2	37	19	48.65	22	25	31	40.54	32.43	16.22
3	36	17	52.78	20	22	27	44.45	38.89	25.00
4	34	15	55.88	17	19	24	50.00	44.12	29.41
5	33	9	72.73	12	16	18	63.64	51.52	45.45

Table 2. Effect of the aqueous extract and dilutions of *Allium cepa* on the number of local lesions developed by PYV infection on *Chenopodiumamaranticolor in vitro*

Time (Days)	Mean Number of Local Lesions								
	Control	Aqueous Extract	% Inhibition	Dilutions			% Inhibition		
				10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
1	39	32	17.95	33	35	38	15.38	10.26	2.56
2	37	29	21.62	31	33	35	16.22	10.81	5.40
3	36	27	25.00	28	30	32	22.22	16.67	11.11
4	34	24	29.41	25	27	30	29.41	20.59	11.76
5	33	19	42.42	21	23	24	36.36	30.30	27.27

Allium cepa possess an inhibitory effect against PVY infectivity *in vivo.* It has been observed that higher percentages of inhibition were achieved via the pre-inoculation treatment rather than post-inoculation. This is true for both *Artemisia absinthium* and *Allium cepa*. The pre-inoculation percentage of inhibition against PVY infectivity with aqueous extract of *Artemisia absinthium* was 54.54% after 5 days, compared with 45.45% post-inoculation after the same period. As for the PVY inhibitory of the aqueous extract of *Allium cepa*, the pre-inoculation percentage was 36.36% compared to 24.24% post-inoculation. Again, as the dilutions increased from 10^{-1} to 10^{-3} the inhibitory effect against PVY infectivity decreased significantly compared to the aqueous extract for both plant extracts. Again, figures 3 and 4, respectively, demonstrate that *Artemisa absinthium* was more effective at reducing the number of local lesions produced by PVY infection on *Chenopodium amaranticolor* than *Allium cepa.*

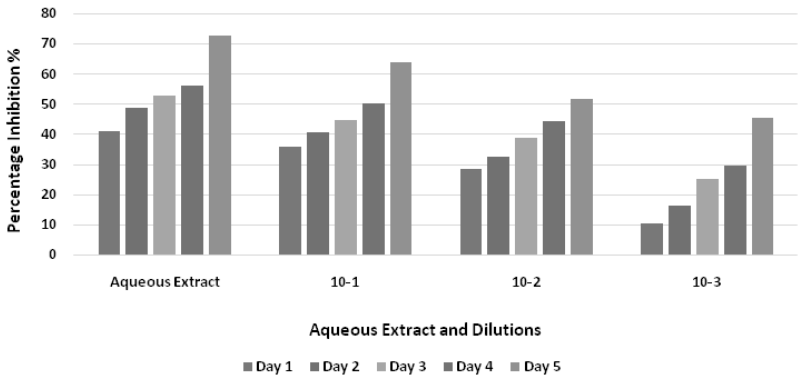

Fig. 1. Percentage inhibition for the aqueous extract and dilutions of *Artemisia absinthium* during 5 days*in vitro*

Table 3. Effect of the aqueous extract and dilutions of *Artemisia absinthium* on the number of local lesions developed by PYV infection on *Chenopodium amaranticolor in vivo*

Time (Days)	Control	Number of Local Lesions (Mean)														
		Pre-Inoculation							Post-Inoculation							
		Aqueous Extract	% Inhibition	Dilutions			% Inhibition			Aqueous Extract	% Inhibition	Dilutions			% Inhibition		
				10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}			10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
One	39	27	30.77	30	33	37	23.08	15.38	5.13	29	25.64	30	34	38	23.08	12.82	2.56
Two	37	23	37.84	26	31	35	29.73	16.22	5.41	26	29.73	29	31	36	21.62	16.22	2.70
Three	36	19	47.22	23	28	31	36.11	22.22	13.89	25	30.56	26	30	35	27.78	16.67	2.78
Four	34	16	52.94	21	24	29	38.24	29.41	14.71	22	35.29	23	27	32	32.35	20.59	5.88
Five	33	15	54.54	18	23	26	45.45	30.30	21.21	18	45.45	21	26	30	36.36	21.21	9.09

Table 4. Effect of the aqueous extract and dilutions of *Allium cepa* on the number of local lesions developed by PYV infection on *Chenopodiumamaranticolor in vivo*

Time (Days)	Control	Number of Local Lesions (Mean)														
		Pre-Inoculation							Post-Inoculation							
		Aqueous Extract	% Inhibition	Dilutions			% Inhibition			Aqueous Extract	% Inhibition	Dilutions			% Inhibition		
				10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}			10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
One	39	33	15.38	34	36	37	12.82	7.69	5.13	35	10.26	36	36	38	7.69	7.69	
Two	37	30	18.92	31	34	36	16.22	8.11	2.70	32	13.51	34	36	36	8.11	2.70	
Three	36	26	27.78	29	32	35	19.44	11.11	2.78	31	13.89	33	34	35	8.33	5.56	
Four	34	24	29.41	26	29	33	23.53	14.71	2.94	28	17.65	30	32	34	11.76	5.89	
Five	33	21	36.36	23	27	30	30.30	18.18	9.10	25	24.24	27	31	33	18.18	6.06	

Fig. 2. Percentage inhibition for the aqueous extract and dilutions of *Allium cepa* during 5 days*in vitro*

Fig. 3. Percentage inhibition for the pre-inoculation and post-inoculation treatment with the aqueous extract and dilutions of *Artemisia absinthium* during 5 days *in vivo*

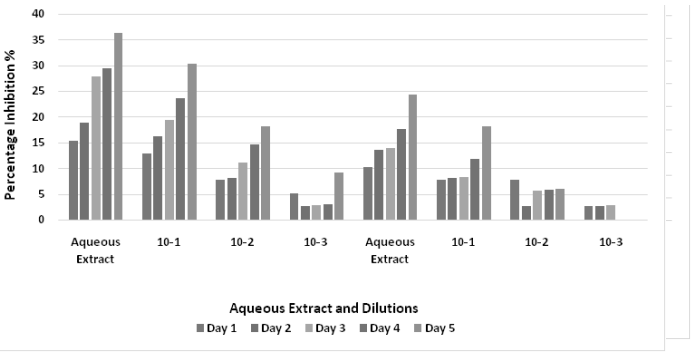

Fig. 4. Percentage inhibition for the pre-inoculation and post-inoculation treatment with the aqueous extract and dilutions of *Allium cepa*during 5 days *in vivo*

DISCUSSION

Similar studies have identified antiviral properties in various plant extracts and lower percentages of inhibition in in vivo tests than in vitro tests[21,30]. The inhibitory effect of *Artemisia absinthium* and *Allium cepa* could be due to various reasons. Oils of Artemisia plants have been observed to have an inhibitory effect on bacterial growth (*Escherichia coli, Staphylococcus aureus*, and *Staphylococcus epidermidis*), yeasts (*Candida albicans, Cryptococcus neoformans*), and dermatophytes (*Trichophytonrubrum*, *Microsporumcanis*, and *Microsporumgypseum*), *Fonsecaeapedrosoi* and *Aspergillus niger*[31,32,33,34].

In one study, the antibacterial and antifungal activities of the essential oils isolated from *Artemisia dracunculus*, *Artemisia absinthium*, *Artemisia santonicum*, and *Artemisia spicigera* oils were also evaluated. In general, the oils exhibited potent antifungal activity at a wide spectrum on the growth of agricultural pathogenic fungi. Among the oils, the weakest antifungal activity was shown by the oil of *A. dracunculus*.

In many cases, the oils of *A. absinthium*, *A. santonicum*, and *A. spicigera* completely inhibited the growth of some fungal species. As compared with antibacterial activities of all of tested oils, *A. santonicum* and *A. spicigera* oils showed antibacterial activities over a very wide spectrum. However, the essential oils tested showed lower inhibition zones than the inhibition zones of penicillin[35].

Furthermore, extended research has been carried out on the antimicrobial properties of herbs belonging to the family *Asteraceae*, trying to establish their potential use in natural pest control, in addition to human medicinal applications. For testing and quantifying antibacterial activity, most often standard microbial protocols are used. In this study the aggregate bactericide effect of four species (*Artemisia absinthium L., A. vulgaris L., Chrysanthemum leucanthemum L. and Achilleamillefolium L.)* was screened using the *Vibrio fischeri* bioluminescence inhibition bioassay. The two *Artemisia* species which have well-established use as antibacterials, exerted the highest toxicity[36].

Allium cepa (onion) belongs to the genus *Allium* which comprises nearly 550 species. Onion is important both as a food plant and as a drug in folk medicine. It has been used in the latter since the middle ages and throughout history in the Arab world. Onion bulbs contain a large number of organic sulphur compounds including Trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, Spropylcycteinesulfoxides and cycloallicin, flavinoids, phenolic acids, sterols including cholesterol, stigma sterol, b-sitosterol, saponins, sugars and a trace of volatile oil compounds mainly of sulphur com- pounds[37]. Many parts of the plant include compound that have been established to possess antibacterial, antiviral, antiparasitic and antifungal properties[38,39]. It has been suggested that the sulphur compounds may be behind the antiviral properties of the plant, in addition to strong phenolic and steroidal compounds[40]. Onion has also been noted for its antioxidant, anticarcinogenic, antimutagenic, antiasthmatic, immunomodulatory and prebiotic activities[41]. Other research observed that the inhibitory effects that onion possesses may be due to the presence of the flavonoids quercetin and kaempferol[42].

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