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Evaluation of the Inhibitory Effect of Some Plant Crude Extracts Against *Albugo Candida*, the Causal Agent of White Rust

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***Abstract*—**White rust, caused by *Albugo candida*, is the most destructive foliar diseases of persian cress, *Lepidium sativum* in Iran. Application of fungicide is the most common method for the disease control. However, regarding the problems created by synthetic pesticides application, environmentally safe methods are needed to replace chemical pesticides. In this study, the antifungal activity of plant natural extracts was investigated for their ability to inhibit zoospore release from sporangia of *A. candida*. The crude extract of 46 plants was obtained using methanol. The inhibitory effect of the extracts was examined by mixing the plant extracts with a zoosporangial suspension of *A. candida* (1×10^6 spore/ml) at three concentrations, 250, 100 and 50 ppm. The experiments were conducted in a completely randomized design, with three replicates. The results of the experiment showed that three out of 46 plants species, including, *Rhus coriaria,* A*nagallis arvensis* and *Mespilus germanica* were completely inhibit zoospore release from zoosporangia of *Albugo candida at* concentration of 50 ppm.

***Keywords*—**white rust, plant extract**,** *Rhus coriaria,* A*nagallis arvensis* and *Mespilus germanica*

I. Introduction

WHITE rust, caused by *Albugo candida* (Pers. ex Hook.) Kunze, is the most serious and destructive foliar diseases of persian cress, *Lepidium sativum* L. in Iran [2]. The pathogen can infect all aboveground parts of the plant, producing white blisters [6]. In Iran, sever outbreaks occur during the spring and fall months [2]. The disease mainly controlled by fungicide application. However, regarding problems created by synthetic pesticides application, alternative safe methods are needed for disease control. Plant extracts are alternative source of natural pesticide for controlling plant diseases. The efficiency of the plant crude extract against plant pathogenic fungi has been already reported [1, 3, 4, 5]. The objective of the present study was to investigate the effect of some plant crude extracts for their ability to inhibit zoospore release from sporangia of *Albugo candida*.

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II. Materials and Methods

A. Plant materiales

Forty six plant species were collected from the various parts of Kermanshah Province and Hamadan Province. The plants were identified based on morphological characteristics and a specimen of each species was kept in the herbarium of Campus of Agriculture and Natural Resources, Razi University, Kermanshah, Iran. Plant parts were cleaned, air dried in the shade and ground to a fine powder with a coffee grinder.

B. Inoculum production

An isolate of *A.candia* was originally recovered from a Persian cress field in Kermanshah and inoculated on persian cress seedling to produce sufficient fresh inoculum for each *in vitro* experiment.

C. Preparation of crude extract

Methanolic extract was obtained by adding 100 ml methanol to 5g ground sample and shaking on an orbital shaker at 300 rpm for 24 hours. Then, thirty milliliter of distilled water and 100 ml n-hexane was added to 70 ml methanolic extract after filtrating and let the mixture to shake for 2 hours. Methanolic phase was then separated and concentrated with a rotary evaporator [1].The obtained extracts were stored at a temperature of 4 ˚C until they were used in the experiment.

D. Antifungal activity test

For each experiment, infected leaves were collected from heavily infected plants and fresh zoosporangia were harvested by washing with sterile distilled water. Resulting suspension was filtered through several layers of muslin. The number of zoosporangia was counted using a haemocytometer and adjusted to a concentration of 1×10^6 spore/ ml. To assess the effect of plant crude extracts on zoospore release from sporangia of *A. candida*, zoosporangial suspension was mixed with appropriate quantity of plant extract to achieve favorable concentrations (250, 100 and 50 ppm).

To promote zoospore release, zoosporangial suspension was transferred to a refrigerator at 8°C and then was incubated at room temperature for 30 min. The number of motile zoospores was counted using a haemocytometer. Counting the zoospores repeated twice and the average were considered. The inhibition percentage was calculated based on following equation:

Inhibition percentage =(C-T/C)/100

Where C is the mean number of released zoospore in control and T is the mean number of released zoospore in each treatment.

The experiments were conducted in a completely randomized design, with three replicates. The inhibitory effect of the plant extracts that completely prevent zoospore release at concentration of 250 ppm were evaluated at lower concentrations, 100 and 50 ppm.

III. Results and Discussion

The results of analysis of variance for the extract concentration of 250 ppm showed significant variation among extracts for the inhibitory effects on the zoospore release of *A.candida*. Mean comparison of treatments done by Duncan's test ($P \leq 0.05$) indicated that the inhibitory effect of 32 out of 46 was categorized in a group with the highest inhibition (Table 1.). Among this group of treatments, 17 were completely inhibited the release of zoospore at this concentration. Therefore, to select the best, zoospores were exposed to these extracts at concentration of 100 ppm. The significant variation for inhibitory effect of extracts was observed and the results showed that 12 out of 17 were classified in the group "a". Nine of them completely inhibited the release of zoospores. So, these 9 extracts were also tested in the third experiment at the concentration of 50 ppm. Finally, 5 of those were non-significantly inhibited well the release of zoospores. Three of those that completely inhibited the release of zoospore were selected for further research on the *in vivo* experiments (Table 1.). These selected plant extracts were methanolic extracts of *Rhus coriaria*, A*nagallis arvensis* and *Mespilus germanica.* It could be concluded that plant extracts collected from the west of Iran are the valuable sources of natural antifungal substances against White rust, and therefore the research needs to be developed in this field.

TABLE I
THE EFFECT OF THE PLANT METHANOLIC EXTRACTS AT DIFFERENT CONCENTRATIONS ON THE RELEASE OF ZOOSPORE FROM ZOOSPORANGIA OF *ALBUGO CANDIDA*

No	Plant	Family	Part used	Inhibitory Effect (%)		
				250 ppm	100 ppm	50 ppm
1	*Oliveria decumbens*	Apiaceae	Shoot	63.6 ^{abcde}	-	
2	*Acroptilon repens*	Asteraceae	Whole plant	68.9 ^{abcde}		
3	*Allium heamanthoides*	Liliaceae	Corm	100.0 ^{a}	61.3^{c}	
4	*Rhus coriaria*	Anacardiaceae	Shoot	100.0 ^{a}	100.0 ^{a}	100.0 ^{a}
5	*Purtulaca oleracea*	Purtolaceae	Whole plant	62.0^{abcde}		
6	*Capsella bursa- pastoris*	Cruciferae	Whole plant	67.4 ^{abcde}		
7	*Xanthium strumarium*	Asteraceae	Shoot	63.7 ^{abcde}		
8	*Rosmarinus officinalis*	Lamiaceae	Shoot	100.0 ^{a}	100.0 ^{a}	50.3^{d}
9	*Verbascum nigrum*	Scrophulariaceae	Shoot	100.0 ^{a}	100.0 ^{a}	99.7 ^{a}
10	*Eugenia caryophyllata*	Caryophyllaceae	Shoot	100.0 ^{a}	100.0 ^{a}	66.7 ^{c}
11	*Carum copticum*	Apiaceae	Seed	100.0 ^{a}	62.1^{c}	
12	*Pinus eldarica*	Pinaceae	Seed	100.0 ^{a}	100.0 ^{a}	89.9 ^{ab}
13	*Alhaji psudoalhaji*	Fabaceae	Shoot	71.4 ^{abcd}		
14	*Physalis alkekengi*	Solanaceae	Shoot	63.2^{abcde}		
15	*Borago officinalis*	Boraginaceae	Flower	69.2 ^{abcde}		
16	*Lavandula officinalis*	Lamiaceae	Shoot	54.3 ^{abcdef}		
17	*Achillea millefolium*	Asteraceae	Whole plant	100.0 ^{a}	52.9^{d}	
18	*Ferulago angulata*	Apiaceae	Shoot	45.8 ^{cdefgh}		
19	*Vaccaria pyramidata*	Caryophyllaceae	Shoot	100.0 ^{a}	86.4^{ab}	
20	*Centaurea depreses*	Asteraceae	Whole plant	36.9 ^{efghi}		
21	*Consolida orientalis*	Ranunculaceae	Whole plant	50.7 ^{bcdefg}		
22	*Tanacetum* sp.	Asteraceae	Whole plant	88.4 ^{ab}		
23	*Citrullus colocynthis*	Cucurbitaceae	Shoot	53.8 ^{abcdef}		
24	*Eucalyptus sp.*	Myrtaceae	Leaf	43.5 ^{defgh}		
25	*Cucumis melo var.dudiam*	Cucurbitaceae	Shoot	49.9 ^{bcdefg}		
26	A*nagallis arvensis*	Myrsinaceae	Shoot	100.0^{a}	100.0 ^{a}	100.0 ^{a}

No	Plant	Family	Part used	Inhibitory Effect (%)		
				250 ppm	100 ppm	50 ppm
27	*Stachys inflata*	Lamiaceae	Shoot	36.7 ^{efghi}		
28	*Onosma* sp.	Boraginaceae	Whole plant	58.9^{abcde}		
29	*Tecurium* sp.	Lamiaceae	Whole plant	45.1 ^{cdefgh}		
30	*Linaria* sp.	Scrophylariaceae	Shoot	63.5^{abcde}		
31	*Tragopogon graminifolius*	Asteraceae	Leaf	100.0 ^{a}	79.1^{b}	
32	*Euphorbia* sp.	Euphorbiaceae	Whole plant	100.0 ^{a}	65.6^{c}	
33	*Melia azedarach*	Meliaceae	Shoot	43.4 ^{defgh}		
34	*Hypericum perforatum*	Hypericaceae	Shoot	100.0 ^{a}	100.0 ^{a}	82.0^{b}
35	*Dorema aucheri*	Apiaceae	Leaf	100.0 ^{a}	89.1^{ab}	
36	*Mespilus germanica*	Rosaceae	Leaf	100.0 ^{a}	100.0 ^{a}	100.0 ^{a}
37	*Tribulus terrestris*	Zygophyllaceae	Shoot	83.6 ^{abc}		
38	*Dracocephalum moldavica*	Lamiaceae	Shoot	68.9 ^{abcde}		
39	*Cuminum cyminum*	Apiaceae	Shoot	29.6 ^{fghi}		
40	*Convulvulus arvensis*	Convulvulaceae	Shoot	20.6 ^{i}		
41	*Alisma samuele* var *orientale*	Alismaceae	Shoot	25.7^{hi}		
42	*Allium noeanum*	Liliaceae	Shoot	27.9 ^{ghi}		
43	*Foeniculum vulgare*	Apiaceae	Seed	23.2 ^{i}		
44	*Thymus* sp.	Lamiaceae	Shoot	44.5 ^{cdefgh}		
45	*Rubia tinctorum*	Rubiaceae	Shoot	100.0 ^{a}	85.4^{ab}	
46	*Zingiber officinale*	Zingiberaceae	Rhizome	100.0 ^{a}	100.0 ^{a}	84.2 ^{b}

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