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Antifungal Potential and Characterization of Plant Extracts against *Fusarium fujikuroi* on Rice

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ABSTRACT

Bakanae rice disease caused by fungus *Fusarium fujikuroi* Nirenberg (*F. fujikuroi*) and occurs in the most growing rice areas of the world. Transmission of this disease by soil and seed, so seed addressing by different material is useful to control bakanae disease. This study is focused on antifungal activity for some plant extracts *i.e. Ammi visnaga (A. visnaga), Eucalyptus globulus* (*E. globulus*), *Artemisia judaica (A. judai)* and *Coriandrum sativum* (*C. sativum*) on the linear growth of *F. fujikuroi*. Identification of chemical compounds was done for two plant extracts *A. visnaga* and *E. globulus* by qualitative and quantitative phytochemical screening, and gas chromatography-mass spectrometry (GC-MS) analysis. Seeds was treatments with plant extracts with different concentrations under greenhouse condition. Changes in seedling content for enzymes such as peroxidase (POX), polyphenol oxidase (PPO) and hydrogen peroxide (H_2O_2) after 7 and 14 days of treatments by plant extracts has been observed. Management bakanae disease under field condition by seed treatment with plant extracts. The results indicate that *A. visnaga* was the most effective on the linear growth with concentration 500 ppm. The obtained results from qualitative phytochemical tests for *A. visnaga* indicates presence of coumarins, tannins, saponin, terpenoids, flavonoids and steroids and absence of phenols. *E. globulus* found tannins, phenols, saponin, terpenoids, flavonoid and absences of coumarins and steroids. Tannins and terpenoids were the highest quantitative phytochemical constituent determined in *A. visnaga*. While phenols and flavonoids were the most active phytochemical constituents determined in *E. globulus*. The chemical constituents for GC-MS analysis of *A. visnaga* were benzene methyl, khellin, visnagin and vitamin E. While, *E. globulus* has eucalyptol, terpinen, ellagic acid and gallic acid. Under greenhouse condition, *A. visnaga* was the most effective in reducing the number of death and number of infected seedling, increasing the germination % at different concentrations compared with other treatments. POX, PPO and H_2O_2 were induced in inoculated seedling compared with the un-inoculated seedling. Seed treatments with *A. visnaga* and *E. globulus* produced the highest enzymes increase and decreased H_2O_2 content in seedling. Under field condition, there are no significant difference between *A. visnaga* and Rhizolex-T 50% as seed treatment in reduction of disease incidence or disease severity of bakanae in 2017 and 2018 growing seasons. Grain yield was increased in treated plant with *A. visnaga* and Rhizolex-T 50% in both seasons.

Keywords: Bakanae, Rice, *Fusarium fujikuroi*, plant extracts, GC-MC

INTRODUCTION

The causal fungus of bakanae disease is Fusarium fujikuroi Nirenberg (F. moniliforme Sheld.), with telomorph Gibberella fujikuroi Sawada, (Wollenweber), and also named (foolish seedling). Recently this disease become important and is widely distributed in Asia, Africa, North America, Italy (Ou, 1985 and Prá *et al*, 2010) and other rice growing countries of the world (Singh and Sunder, 2012). The disease during rice plant growth stages could be reached up to 70% for yield losses under outbreaks (Ou, 1985). The symptoms started from rice seedling to maturing. The infected plants suffer from abnormal elongation, thin noodle, chlorosis, empty panicles with poor grain ripening with foot and stem rot (Sun and Snyder, 1981) with ultimate effect on yield and seed quality which have been recorded from different localities of the world (Webster and Gunnell, 1992; Desjardins *et al*, 2000). Rice plants will die in extreme infection because they are no longer strong enough to support their own weight (Hwang *et al*, 2013). Bakanae is considered both as seed-borne and soil-borne disease. The disease is transmitted by seeds and some seed soaking by chemicals are usually used to control the disease. Chemical fungicides affected on human health and environment. Use of plant extracts has become more activate to control plant diseases. Some researcher investigators the antifungal activity of different plant extracts against a number of plant pathogens (Hasan *et al*, 2005; Yang and Clausen, 2007). Miah *et al* (1990) indicated that the inhibitory effect of plant extracts against F. moniliforme. Thus, it is necessary to find out the plant parts which have antifungal principles against F. moniliforme. Yasmin *et al* (2008) evaluated fifty five plant extracts against F. moniliforme. Extracts of 17 plants showed varied degrees of inhibitory effects on the test pathogen. Abd El Khair and Nadia (2011) detected 22 different plant extracts against two soil pathogens, F. solani and Rhizoctonia solani, and their effect on the linear growth. Satish *et al* (2009) revealed that 12 plants have recorded significant antifungal activity against F. spp and that these plants could be exploited for eco-friendly management.

The aim of the present work to study the antifungal activity of plant extracts against F. fujikuroi and identification of some component in those plant extract under study. In addition how these plant extracts can be used for the management of bakanae disease under greenhouse and field condition.

MATERIALS AND METHODS

Laboratory, greenhouse and field studies: Laboratory and greenhouse experiments were conducted at the Rice Pathology Department, while field experiments were performed at Sakha Agricultural Research Station farm, Egypt.

Fungus preparation: Bakanae fungus was isolated from infected rice plants of Sakha 101 rice cultivar during 2016 season from Kafr El-Sheikh governorate. The purification was done by hyphal tip technique according to Hansen (1926). The pathogen F. fujikuroi was identified according to the morphological characteristics and microscopic examination at plant pathology laboratories using the key of imperfect fungi Barnett and Hunter (1972); Nelson *et al* (1983) and Summerell *et al* (2003). The pathogen was cultured on potato dextrose agar (PDA) culture medium at 26±2^{o}C until the whole surface of the plate was covered with mycelium. Some of this plates was added 10ml of sterilized water to obtained inoculum of spore suspension. Mycelia mats were gently scraped by spatula and filtered through cheese cloth. Spore suspension was adjusted to (5 X10^{5}/ml).

Antifungal activity assay of plant extracts against *F. fujikuroi:*

Plants parts of A. visnaga (Khella), E. globulus (Blue gum), A. judaica (Artemisia) and C. sativum (Coriander) were extracted by ethanol solution according to (Mangamma and Srevamulu, 1991). Plant extracts were filtered through sterilized membrane (0.45µm mesh). The clear filtrates were incorporated into melted PDA medium just before solidification at the different concentrations 250, 500, 750, 1000 and 1250 ppm, and poured into petri dishes (9 cm in diameter). Plates were inoculated at the center with 5mm culture discs of F. fujikuroi (7 days old) and incubated at 26^0±2C. Fungal growth plates without any plant extracts filtrate were used as control. Linear growth of the fungus was determined daily by measuring growth diameter in each of the three replicate plates until control plate free from the plant extracts reached the edges. Percentage of reduction in colony diameter was calculated for each treatment according to Aurangzeb *et al* (2003).

$$R = \frac{C - T}{C} \quad X\ 100$$

R= percent of inhibition of the fungal growth
C= linear growth of untreated plates.
T= linear growth of treated plates with the plant extracts.

Qualitative and quantitative phytochemical screening:

The ethanol extraction of A. visnaga and E. globulus were explored by qualitative preliminary phytochemical tests and quantitative analysis of the phytochemical constituents determined for coumarins as described by Kakáč, *et al*, 1962 and tannins, phenols, saponin, terpenoids, flavonoids and steroids as described by (Edeoga *et al*, 2005).

Gas chromatography-mass spectrometry (GC-MS):

The chemical constituents of plant extracts filtrate of both tested *A. visnaga* and *E. globulus* were identified using GC-MS system (Agilent Technologies). GC-MS equipped with gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, and Cairo, Egypt.

Evaluation of plant extracts on bakanae disease under greenhouse condition: Plant extracts i.e A. visnaga, E. globulus, A. judaica (L) and C. sativum were used each in different concentrations 500, 750 and 1000 ppm, to test their effect against F. fujikuroi. Seeds of Sakha 101 rice cultivar were soaked in the plant extracts at the three mentioned concentrations for 24hr, then soaked in the pathogen spore suspension (5 x 10^5 spores/ml) for another 24 hr. The seed treatment by plant extracts seeded in plastic pots (15cm diameter), as 50 seeds / pot. For check fifty seeds were soaked in the same fungal spore suspension and other control seeds were soaked in water. The pots were kept in the greenhouse at 30±5°C and fertilized one times with urea 46.5% N at 3g/pot. Three replicates were used for each treatment. After 7 days of sowing the germination percent was recorded. While, number of death seedling, number of infected seedling and seedling height were recorded after 30 days of sowing.

Biochemical analysis: The activities of some defense-related enzymes were assessed in the rice seedling. PPO, POX and H_2O_2 contents were determined as previously describe by Matta and Dimond (1963), Allam and Hollis (1972), Srivastava (1987) and Velikova *et al*, 2000 respectively.

Effect of seed treatments with plant extracts on bakanae disease under field conditions:

This experiment was carried out under field condition during growing seasons of 2017 and 2018 on Sakha 101 rice cultivar (the most susceptible cultivar to rice bakanae disease). Four plant extracts i.e. *A. visnaga* (750ppm), *E. globulus* (1000 ppm), *A. judaica (L) (*1250ppm*) and C. sativum (*1000ppm), were used as seed treatments. Fungicide Rhizolex-T 50% (Thiram 30% + Tolclofos-methyl 20%) WP (2g/L) was used for comparison. Seeds were soaked in each treatment for 24 hr and then soaked another 24 hr in spore suspension of *F. fujikuroi* (5 x10^5 spore/ml). The control treatment was soaked in the same spore suspension and other control soaked in water. All seeds incubated for another 48 hr. and sowing directly in the permanent field with randomized complete block design with three replicates for each treatment.

Disease assessment: - Disease symptoms were started at seedling stage. Disease incidence and severity percentages were assessed and recorded at 30 days after sowing dates according to Teng and James (2001) with slight modifications:

$$\text{Disease Incidence (\%)} = \frac{\text{Total number of infected plants/ m}^2}{\text{Total number of plants/ m}^2} \quad X\ 100$$

$$\text{Disease severity (\%)} = \frac{\text{*Area of infected plants /25 hills}}{\text{Total area of plants/ 25 hills}} \quad X\ 100$$

***Area of infected plants:** area of missing hills as a result of died plants due to infection.

Data Analysis: Data were statistically analyzed using standard statistical analysis with MSTATC. in the table of main treatments, Duncan′s T. (1955) was used to compare the significantly different averages.

RESULTS AND DISCUSSION

Antifungal activity of plant extracts on *F. fujikuroi*:

Plant extracts of *A. visnaga, E. globulus, A. judaica* and *C. sativum* were tested for their effect on *F. fujikuroi* linear growth in petri dishes. Data presented in Table 1 indicate that all tested materials significantly reduced the linear growth of *F. fujikuroi.* The effect was obviously increased by increasing the concentrations of plant extracts from 250 to 1250 ppm. The obtained data showed that the extract of *A. visnaga* was the most effective in decreasing linear growth while it completely inhibited growth of the fungus at concentration of 500 ppm Table 1.

Some medicinal plants have an effect as an antifungal against phytopathogenic fungi. This antifungal activity may be attributed to one or more of the respective bioactive compounds, such as quinines, phenols, tannins and flavonoids. Their antifungal activities due to different mechanisms includes; disruption of cell wall/ membrane integrity (Cho *et al*, 2013), inhibition of enzyme activities (Muhsin *et al*, 2001), induction of oxidative stress (Lemar *et al*, 2005), DNA damage (Cardoso- Lopes *et al*, 2008), inhibition of protein synthesis (Upadhyay *et al*, 2015), and/or down-regulation of the expression of virulence- or toxin-related genes (Yin *et al*, 2015). Younes *et al* (2018) recorded that khella as methanol extract showed the higher activity to inhibit mycelial growth of *R. solani*. El-Mougy and Abdel-Kader (2007) reported the antifungal activity of the methanol

extract from khella at a concentration of 8% prove higher activity to inhibit mycelial growth against *Alternaria solani, F. solani, Macrophomina phaseolina* and *R. solani*. The results from the GC-MS analysis of the *A. visnaga* revealed the presence of 20 chemical compounds. The major antifungal constituents included; khellin (Arif *et al*, 2009). The antifungal activity of the khella extract can probably be attributed to the synergistic effects of these fungitoxic compounds. The antifungal mechanisms that may be utilized by coumarins and phenolic acids include; disruption of cell membranes, suppression of cell wall formation and the mitochondrial dysfunction (Freiesleben and Jäger, 2014). Lee *et al* (2007) recorded that three pathogenic fungi such as *Colletetricum gloeosporioides, R. solani and F. oxysporum* were affected by *Tymus vulgaris* as essential oil and inhibited mycelial growth for these pathogens. *E. globulus* as essential oil have antifungal activity against pathogenic fungi on tomato fruits. Because some chemical constituents found in *E. globulus* such as eucalyptol (1,8-Cineole) and Terpinen-4-ol by GC-MS analysis and these chemical constituents could be responsible for the antifungal activities (Chiamaka *et al*, 2016).The oil has 1.8-cineole component known to induce antimicrobial activity (Sivropoulou *et al*, 1997). Pinene-type monoterpene hydrocarbons are well known chemicals having antimicrobial potentials (Dorman and Deans, 2000).

Table 1. Antifungal activity of plant extracts on the linear growth of *F. fujikuroi*

Plant extracts	Conc./ ppm	Linear growth (cm)	Reduction %
Ammi visnaga	250	1.20^{e}	91.76
	500	0.5^{f}	100.0
	750	0.5^{f}	100.0
	1000	0.5^{f}	100.0
	1250	0.5^{f}	100.0
Eucalyptus globulus	250	2.50^{de}	76.47
	500	1.40^{e}	89.41
	750	0.50^{f}	100.0
	1000	0.50^{f}	100.0
	1250	0.50^{f}	100.0
Artemisia judaica	250	7.92^{b}	12.70
	500	7.16^{b}	21.64
	750	5.96^{c}	35.76
	1000	4.66^{d}	51.05
	1250	0.53^{f}	99.64
Coriandrum sativum	250	2.30^{de}	77.64
	500	1.83^{e}	84.43
	750	1.80^{e}	84.70
	1000	0.80^{f}	96.47
	1250	0.5^{f}	100.0
Rhizolex-T 50%	2000	1.67e	86.23
Control	-	9.00a	-

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Qualitative and quantitative phytochemical screening

The qualitative phytochemical constituents of *A. visnaga* as shown in Table 2 indicate the presence of coumarins, tannins, saponin, terpenoids, flavonoids and steroids and absence of phenols. As well as, *E. globulus* has tannins, phenols, saponin, terpenoids, flavonoid but coumarins and steroids are absent. Tannins and terpenoids were the highest quantitative phytochemical constituent determined on ethanol extract of *A. visnaga.* While, phenols and flavonoids were the most active phytochemical constituents determined in *E. globulus* show in Table 3. Coumarins (1,2-benzopyrones) are known phytoalexin and produced in higher plants rich from the phenylpropanoid pathway. They contribute essentially to the persistence of plants being involved in processes such as regulation of oxidative stress, defense against phytopathogens, response to abiotic stresses and probably hormonal regulation (Bourgaud *et al*, 2006). Some researcher studies on the phytochemistry of *A. visnaga* and found the existence of chemical compounds such as flavonoids, pyrones, saponins and essential oils (Eldomiaty, 1992). Tannins also precipitate the microbial proteins, thus making the nutritional proteins unavailable for microbial growth (Varghese *et al*, 2009).

Table 2. Qualitative phytochemical screening of both plant extract of *Ammi visnaga* and *Eucalyptus globulus*

Compound	*A. visnaga*	*E. globulus*
coumarins	+	-
tannins	+	+
saponin	+	+
phenols	-	+
terpenoids	+	+
flavonoids	+	+
steroids	+	-

Table 3. Quantitative phytochemical screening of both plant extract of *Ammi visnaga* and *Eucalyptus globulus*

Compound	*A. visnaga*	*E. globulus*
	Phyto-constituents (mg/100 g)	
Coumarins	50.00	0.000
Tannins	137.0	45.00
Phenols	0.000	145.25
Saponin	54.00	35.400
terpenoids	98.40	38.88
flavonoids	22.40	89.45
steroids	12.400	0.000

Gas chromatography-mass spectrometry (GC-MS):

Through, using a GC-MS system twenty compounds with varying extents were identified in the extraction of *A. visnaga* Table 4. The major chemicals constituents include; benzene methyl (11.39), khellin (10.35%), visnagin (10.83%), vitamin E (10.46%) and pentamethoxyflavone (7.05%). While, the other constituents including Edulisin, 8-methyl-2-oxo-2H,8H-benzo[1,2-b:3,4-b']dipyran-9,10-diyl-10-acetate-9-(2-methyl), edulisin III, 1,3,5-cycloheptatriene, 7-ethyl-, p-xylene, heptane, 2,5-dimethyl, and benzene methyl existed in intermediate levels. The rest constituents were found at minor percentages. Regarding *E. globulus*, twenty one chemical constituents were identified in their culture filtrate Table 4. The major constituents include eucalyptol (20.67%), terpinen (20.08%), ellagic acid (8.44%) and gallic acid (7.53%). Other components include, Benzene methyl, Benzene, 1-methyl-3-propyl, Benzene,1-methyl-2-(1-methylethyl) and Oleic acid existed in intermediate levels. The rest compounds were found in lowest percentages. These findings are in agreement with Al-Snafi (2013) who recorded that *A. visnaga* was contained more than 4% of γ-pyrones and the basic components are khellin and visnagin with percentage up to (0.3–1.2% and 0.05–0.30%, respectively). Also, it's containing more than 18% of fixed oils and pyranocoumarin visnadin about 0.2–0.5 percent as coumarins. *A. visnaga* was contained eleven flavonols, isolated from the different aerial parts of the plant (Kabouche and Jay, 2011). Twenty one compounds of essential oils were

found in GC/MS analysis in fresh aerial parts of *A. visnaga*. These ingredients were identified as essential oil with percent about 97.3 named 2,2-dimethylbutaoic acid, isobutyrate, croweacin, linalool, bornyl acetate and thymol (Khalfallah *et al*, 2011). Also, Chiamaka *et al* (2016) determined 21chemical compounds for eucalyptus (essential oil) by GC-MS. Eucalyptol (synonymous 1,8-Cineole) was the most abundant compounds followed by 1,4-cyclohexadiene,1-methyl-4-(1-methylethyl), terpinen-4-ol and p-Mnth-1-en-8-ol. Boukhatem *et al* (2014) obtained that 1,8-cineole (51.08 %) and α-pinene (24.6 %). Phytochemical analysis for *E. globulus* with different region Uruguay, Cuba, California, Morocco Africa and Argentina by GC-MS showed that the most compound is eucalyptol with percentage 64.5, 77, 86.7, 58 to 82, 48.7 and 50 to 65, respectively as reported by Viturro *et al* (2003), the different percentage of abundance could be attributed to variation in climatic conditions.

Table 4. The chemical composition of both plant extract of *Ammi visnaga* and *Eucalyptus globulus* using GC-MS

Ammi visnaga				*Eucalyptus globulus*			
Peak	***RT**	**Percentage**	**Compound name**	**Peak**	***RT**	**Percentage**	**Compound name**
1	3.008	11.39	Benzene methyl	1	3.088	0.37	α-Pinene
2	3.39	3.45	Heptane, 2,5-dimethyl	2	4.433	0.71	β- -Pinene
3	3.877	7.47	Benzene, 1,2-dimethyl	3	4.549	4.37	Benzene, 1,2-dimethyl
4	4.809	3.94	p-Xylene	4	4.677	0.27	α-Phellandrene
5	4.962	8.04	Benzene, 1,3,5-trimethyl	5	4.809	2.96	Cyclohexene,4-methyl-3-(1-methylethylidene)
6	5.344	0.99	Benzene propyl	6	5.77	3.02	Benzene,1-methyl-2-(1-methylethyl)
7	5.73	1.38	1,3,5-Cycloheptatriene, 7-ethyl	7	5.905	7.23	Benzene, 1-methyl-3-propyl
8	5.055	1.99	Benzene, 1-ethyl-2-methyl	8	5.958	8.66	Benzene methyl
9	5.905	3.38	Benzene, 1,2,4-trimethyl	9	5.995	2.37	Terpinolen
10	6.991	0.42	Benzene, 1-methyl-3-propyl	10	6.112	20.08	Terpinen
11	7.187	0.85	Benzene, 1,4-diethyl	11	6.154	7.53	Gallic acid
12	7.287	0.67	Benzene, 2-ethyl-1,4-dimethyl	12	6.318	0.55	Zingiberene
13	8.235	10.83	Visnagin	13	6.784	1.03	Caryophyllene
14	19.546	10.35	Khellin	14	19.588	2.82	Aromadendrene
15	20.896	6.25	Edulisin III	15	24.375	2.37	α –Farnesene
16	21.071	4.53	8-methyl-2-oxo-2H,8H-benzo[1,2-b:3,4-b']dipyran-9,10-diyl-10-acetate-9-(2-methyl	16	24.624	20.67	Eucalyptol
17	22.453	10.46	Vitamin E	17	26.017	0.49	Ledol
18	24.539	7.05	4',5,6,7,8-Pentamethoxyflavone	18	26.769	1.00	aphthalenemethanol,decahydro-α,4a-trimethyl-
19	25.276	0.91	gamma.-Sitosterol	19	25.276	1.10	n-Hexadecanoic acid
20	25.943	5.65	4'-Heptamethoxyflavone	20	27.574	2.96	Oleic acid
				21	28.183	8.44	Ellagic acid

***Rate time**

Effect of certain plant extracts on controlling rice bakanae disease under greenhouse condition:

Rice seedling of Sakha 101 cv. was artificially inoculated with spore suspension of *F. fujikuroi* (5 x 10^5 condia /ml) after seed treatments with different plant extracts. Data in Tables 5 indicates that all seed treatments showed positive effect in reducing number of death of seedling, number of infected seedling, seedling height and increased germination percent. *A. visnaga* was the most effective to reduce the number of death and number of infected seedlings and increasing the germination percent with different concentrations compared with other seed treatments. There is no significantly difference has been observed between *A. visnaga* and *E. globulus* extracts for decreasing number of deaths at concentration (1000 ppm). While, *C. sativum* extract was the least effective to reduce the number of death of seedling, number of infected seedling and germination percent. Also, no significant difference between all plant extracts in seedling height.

Using plant extracts for management of plant diseases in the later stages are very important to environment and soil. Several investigators studied the antifungal activity of different plant extracts against the number of plant pathogens such as *F. fujikuroi*. Antifungal properties of bark extract of *E. citriodora* against bakanae disease (Yasmin *et al*, 2008). *Lawsonia inermis* and *Asparagus racemosus* have been found an important plant species for their exploitation as potent natural fungi-toxic with broad spectrum activity to controlling bakanae disease of rice (Yasin *et al*, 2003). Essential oils (EOS) from *Cymbopogon citratus*, *Ocimum gratissimum* and *Thymus vulgaris* were found effective against *F. moniliforme* to controlling the seed infection by 95-100%. The antifungal activity of EOs is due to their contents of thymol, terpinene, p-cymene, carvacrol from *O. gratissimum*, linalool from *T. vulgaris* and citral from *C. citrates* (Nguefack *et al*, 2007). Younes *et al* (2018) found that seed addressing of maize in khella at concentration 10, 15 or 20% for 12 hr reduced incidence of Rhizoctonia root rot, percentage of seed rot and pre and post-emergence

damping off the disease under greenhouse conditions. Mohd *et al* (2018) evaluated six plant extracts against rice blast disease as seed treatment. Seed treated with plant extract decreased the blast incidence and increasing the germination of the seeds as compared to control. El-kazzaz *et al* (2015) reported that *Ammi visnaga* was the most active in reducing the infection of rice kernel smut disease.

Table 5. Effect of seed treatments with certain plant extracts on bakanae disease using Sakha 101 cv. under greenhouse condition

Plant extracts	Concentration /ppm	Germination %	NDS*	NIS**	Seedling height
A. visnaga	500	96.67^{a}	12.0^{b-e}	2.00^{cde}	23.67^{bc}
	750	95.67^{a}	7.34^{def}	1.34^{de}	21.00^{bcd}
	1000	93.34^{a}	6.67^{ef}	1.34^{de}	19.34^{bcd}
E. globulus	500	64.00^{c}	18.00^{b}	5.34^{cd}	20.34^{bcd}
	750	72.00^{bc}	16.00b^{c}	2.67^{cde}	19.00^{bcd}
	1000	86.00^{ab}	5.34^{ef}	2.00^{de}	17.67^{cd}
A. judaica	500	56.00^{c}	16.00^{bc}	5.34^{cd}	21.34^{bcd}
	750	58.67^{c}	12.67^{b-e}	4.67^{cd}	20.34^{bcd}
	1000	58.00^{c}	8.00^{c-f}	4.67^{cd}	19.67^{bcd}
C. sativum	500	56.67^{c}	19.34^{ab}	11.67^{b}	25.00^{b}
	750	57.00^{c}	16.67^{bc}	6.67^{c}	22.00^{bcd}
	1000	62.67^{c}	15.34^{bcd}	4.67^{cd}	20.34^{bcd}
Rhizolex-T 50%	2000	96.67^{a}	3.34^{f}	2.34^{de}	18.00^{cd}
Inoculated seeds	-	54.00^{c}	26.67^{a}	38.67^{a}	32.34^{a}
Un inoculated seeds	-	96.67^{a}	4.67^{ef}	00^{e}	15.67^{d}

***NDS: number of death seedling. **NIS: number of infected seedling**
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

Biochemical analysis:

Data presented in Table 6 indicates that inoculated of rice seedling with bakanae pathogen led to a significantly increase in activities of POX and PPO enzymes and H_2O_2 content compared with un-inoculated control seedling when comparing with the seed treatment with the plant extracts. All treatments with plant extracts were significantly induced of activity for both enzymes and reduced the H_2O_2 content in the rice seedling with varied extents. The highest activities of POX and PPO enzymes obtained with *A. visnaga* and *E. globulus* as seed treatments. Also, the result of H_2O_2 content decreased with the same plant extracts. Generally, activity of enzymes increased with increasing concentration of different plant extracts. El-kazzaz *et al*. (2015) found that POX and PPO enzymes were more activate in plants inoculated with the rice kernel smut pathogen. These enzymes increased in inoculated plants treated by the plant extracts compared with untreated ones.

The antifungal activity of medicinal plant extracts against the fungal pathogens may be due to indirect effects on host plants such as induction of host plant immune systems against pathogens (Abkhoo and Jahani, 2017). Also, other responses of medicinal plant include; induced defense-related genes, pathogenesis-related (PR) proteins, accumulation of phytoalexins, and deposition of lignin and programmed cell death (Goel and Paul, 2015). POX considered ones for important group of enzymes involved in plant resistance mechanisms, such as synthesis of phenolic compounds and the formation of structural barriers (Dalisay & Kuć, 1995). Scherer *et al* (2005) found that POX are coded by host plants (as pathogen related proteins) because his response to pathological or related situations, normally accumulate not only locally in the place of infection but also formed systemically, any kind of infection. Several roles have been attributed to POX in host–pathogen interactions (Kristensen *et al*, 1999). The structural barriers were formatted by polymerization of lignin and suberin (Espelie *et al*, 1986), cross-linking of cell wall protein (Bradley *et al*, 1992) and dimerization of antimicrobial phenols (Martínez-Espinoza *et al*, 2002). Elimination of reactive oxygen species (ROS) is mainly achieved by antioxidant compounds such as PPO and POD. Under stress conditions, a constitutively high antioxidant capacity can prevent damages due to ROS formation (Harinasut *et al*, 2003). Increased PPO activity under stress indicates the ability to oxidize and degrade the toxic substance such as phenolic compounds which are generally accumulated during stress (Weisany *et al*, 2012). PPO plays a role in defence responses to biotic stresses such as direct toxicity of quinones, reduced bioavailability and alkylation of cellular proteins to the pathogen, cross-linking of quinones with protein or other phenolics and forming physical barriers (Castañera *et al*, 1996). The H_2O_2 concentration of leaf tissue was significantly increased with increasing salinity (Weisany *et al*, 2012).

Table 6. Effect of seed treatment with plant extracts on the defense-related enzyme and H_2O_2 content of rice seedling inoculated with *F. fujikuroi*

Plant extracts	Conc. /ppm	PPO (ΔA420 min/1g/1 fresh weight)		POX (ΔA470 min/1g/1 fresh weight)		H_2O_2(ΔA290 min/1g/1 fresh weight)	
		Days after inoculation					
		7 days	14 days	7 days	14 days	7 days	14 days
A. visnaga	500	0.218^{ab}	0.212^{ab}	1.36^{bc}	1.51^{ab}	0.118^{cde}	0.148^{de}
	750	0.228^{a}	0.224^{ab}	1.44^{b}	1.57^{a}	0.117^{cde}	0.116^{e-h}
	1000	0.236^{a}	0.232^{ab}	1.63^{a}	1.65^{a}	0.112^{b-e}	0.112^{fgh}
E. globulus	500	0.181^{abc}	0.207^{ab}	1.210^{ef}	1.27^{cde}	0.142^{b-e}	0.139^{def}
	750	0.203^{ab}	0.214^{ab}	1.25^{de}	1.38^{bc}	0.137^{b-e}	0.129^{d-g}
	1000	0.210^{ab}	0.222^{ab}	1.31^{cd}	1.50^{ab}	0.130^{cde}	0.122^{d-g}
A. judaica	500	0.161^{abc}	0.169^{b}	1.14^{fg}	1.11^{fgh}	0.167^{bcd}	0.185^{c}
	750	0.165^{abc}	0.174^{b}	1.15^{fg}	1.26^{c-f}	0.166^{bcd}	0.152^{d}
	1000	0.174^{abc}	0.193^{b}	1.20^{ef}	1.15^{d-g}	0.147^{cde}	0.147^{de}
C. sativum	500	0.132^{bc}	0.169^{b}	0.92^{ij}	1.00^{gh}	0.220^{ab}	0.242^{b}
	750	0.138^{bc}	0.173^{b}	0.998^{hi}	1.030^{gh}	0.189^{bc}	0.207^{c}
	1000	0.152^{abc}	0.176^{b}	1.070^{gh}	1.14^{efg}	0.177^{bcd}	0.181^{c}
Rhizolex-T 50%	2000	0.172^{abc}	0.317^{a}	1.39^{bc}	1.30^{cd}	0.094^{de}	0.097^{gh}
Inoculated seeds	-	0.108^{c}	0.134^{c}	0.866^{jk}	1.00^{gh}	0.293^{a}	0.294^{a}
Un inoculated seeds	-	0.096^{c}	0.095^{c}	0.776^{k}	0.96^{h}	0.072^{e}	0.085^{h}

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Effect of seed treatments with plant extracts on rice bakanae disease under field condition:

Different plant extracts were studied for their effect on bakanae disease on Sakha 101 rice cv. under artificial infection at Sakha farm during 2017 & 2018 seasons. Rhizolex-T 50% was used as seed treatment at (2g/kg seeds). As well as the untreated seeds were used for comparison. Data presented in Table 7 revealed that there is no significantly difference between plant extract *A. visnaga* and Rhizolex-T 50% in reducing the disease incidence of bakanae as well as the disease severity in 2017 and 2018 growing seasons. While, there is no significantly difference between other treatments in decreasing disease severity and incidence. The grain discoloration decreased by decreasing the disease severity on plant compared with the untreated plant with the tested plant extracts and essential oil. Grain yield was increased in plant treated with *A. visnaga* and Rhizolex-T 50% in both seasons. Also, unfilled grains decreased in plant treated with *A. visnaga* followed by Rhizolex-T 50% in the 2017 growing season. But, in 2018 growing season there are no differences between all treatments.

Table 7. Effect of seed treatments with plant extracts on bakanae disease

Plant extracts	Disease incidence (%)		Severity (%)		Unfilled grain		Discoloration grain		Yield (ton/fed)	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
A.visnaga	1.75^{bc}	1.50^{b}	1.34^{b}	2.67^{bc}	49.34^{e}	68.34^{b}	54.34^{bc}	86.67^{cd}	4.400^{a}	4.580^{a}
E. globulus	1.83^{b}	1.67^{b}	4.00^{b}	2.67^{bc}	104.0^{cde}	104.0^{b}	98.34^{abc}	120.0^{a-d}	3.970^{b}	3.867^{b}
A. judaica	2.25^{b}	2.88^{b}	8.00^{b}	8.00^{b}	146.6^{cd}	123.0^{b}	106.67^{abc}	121.0^{abc}	3.907^{b}	3.853^{b}
C. sativum	2.08^{b}	1.50^{b}	6.00^{b}	8.67^{b}	213.3^{b}	127.0^{b}	114.34^{ab}	134.67^{ab}	3.587^{b}	3.680^{b}
Rhizolex-T 50%	0.92^{bc}	1.05^{b}	1.34^{b}	1.34^{c}	89.34^{de}	103.4^{b}	41.67^{c}	80.00^{d}	4.400^{a}	4.582^{a}
Inoculated seeds	8.58^{a}	8.42^{a}	76.0^{a}	62.6^{a}	313.3^{a}	236.67^{a}	153.34^{a}	159.34^{a}	3.285^{b}	3.390^{b}
Un inoculated seeds	0.00^{c}	0.00^{b}	0.00^{b}	0.00^{c}	167.0^{bc}	114.67^{b}	63.67^{bc}	100.0^{bcd}	3.613	3.817^{b}

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT.

El-kazzaz *et al*. (2015) recorded that plant extracts significantly reduced disease severity of *T. barclayana* the causal fungus of rice kernel smut disease under field condition. Soaked rice seeds with fungicides such as carbendazim or benlate (0.1%) for 8 hr, reduced disease incidence of bakanae rice up to 92%. Also, Propiconazole 25 EC at 0.05% decreased disease incidence of bakanae, but also affected the plant height and grain yield (Bagga and Sharma, 2006). An application rice plant with plant extracts i.e. *A. visnaga*, *Schines terbenthifolium*, *Pancratium martmium*, *Solanum nigrum* and *Cumin cryminum* in the field for two seasons aiming to control rice blast disease (Salem *et al*, 1999).

On the bases of findings in the present study it can suggested to use plants extracts as a possible seed treatment to controlling bakanae disease. Outcomes of the current results can use plant extracts as seed treatments to controlling bakanae disease. Plant extracts are ecofriendly for environmental and have good compound to effective on the bakanae pathogen.

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الإمكانات المضادة للفطريات وتوصيف المستخلصات النباتية ضد الفيوزاريوم فيوجيكورا علي الارز

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قسم بحوث أمراض الأرز- معهد بحوث أمراض النباتات - مركز البحوث الزراعية – الجيزة

يتسبب عن مرض البكانا في الأرز فطر (فيوزاريوم فيوجيكورا) حيث وجد في معظم مناطق زراعة الأرز في العالم. ينقل هذا المرض بالبذره والتربه، لذلك فأن معاملة البذرة بالمواد المختلفه مفيده لمقاومة مرض البكانا. وتركز هذه الدراسة على النشاط المضاد للفطريات لبعض المستخلصات النباتية على النمو الخطي للفطر فيوزاريوم فيوجيكورا. تعريف المركبات الكيميائيه لمستخلصي نبات الخله والكافور من خلال فحص الاختبارات الكميه و النوعيه للمركبات الكيميائيه النباتيه و التحليل اللوني الكتلي بواسطة الغاز GC-MS. تم معاملة الحبوب بواسطة المستخلصات النباتية وهي الخلاء والكافور، والشيح والكزبره بتركيز مختلف تحت ظروف الصوبه الزجاجيه. قياس التغير في محتوي البادرات من الانزيمات مثل البيروكسيديز والبوليفينول اوكسيديز وايضا فوق اكسيد الهيدروجين بعد 7 و14 يوم من المعامله بالمستخلصات النباتيه. التحكم في مرض البكانا علي الارز تحت ظروف الحقل بواسطة معاملة الحبوب بالمستخلصات النباتيه. تشير النتائج إلى أن مستخلص نبات الخله كان الأكثر تأثيرًا على تثبيط النمو الخطي عند التركيز (500 جزء في المليون). البيانات التي تم الحصول عليها من الاختبارات الكيميائية النباتية النوعية لـ مستخلص الخلا وجود الكومارين والتانينات والسابونين والتربينويدات والفلافونويدات والستيرويدات مع غياب الفينولات. اما مستخلص الكافور وجدت التانينات والفينولات والسابونين والتربينويدات والفلافونويد ومع غياب الكومارين والستيرويدات. بينما في التقدير الكمي كانت التانينات والتربينويدات أعلى في مستخلص الخلا . وكانت الفينولات والفلافونويد أكثر المكونات الكيميائية النباتية نشاطًا في مستخلص نبات الكافور. التحليل باستخدام جهاز التقدير اللوني والكتلي بالغاز GC-MS لنبات الخله كانت المركبات الكيميائيه هي ميثيل البنزين، الخللين ، فيسنيجين وفيتامين د. بينما ، يحتوي الكافو على الايوكاليبتول ،تربنين ،حمض الاجيك بنسبة ، وحمض الجالي. تحت ظروف الصوبه الزجاجيه، كان مستخلص نبات الخله هو الأكثر فعالية في تقليل عدد موت البادرات وعدد البادرات المصابة ، وزيادة نسبة الإنبات بتركيز مختلف مقارنة بالمعاملات الأخرى. ازدياد نشاط البيروكسيديز والبوليفينول اوكسيديز و فوق اكسيد الهيدروجين في البادرات الملقحة بالفطر المسبب مقارنة بالبادرات غير الملقحة(السليمه). ثبت أن معاملة البذور بمستخلص نبات الخله و الكافور هي الأعلى زيادة بالنسبة للإنزيمات وانخفضت نسبة فوق اكسيد الهيدروجين في البادرات. تحت ظروف الحقلية ، لا يوجد فروق معنويه بين مستخلص نبات الخله والمبيد الفطري ريزولكس تي 50% كمعامله للحبوب للحد من الإصابة بمرض البكانا وكذلك شدة المرض في موسمي النمو 2017و 2018. تمت زيادة محصول الحبوب في النبات المعامل بـ مستخلص نبات الخله والمبيد الفطري ريزولكس تي 50% في كلا الموسمين.