Antifungal Activity of Crude and Phenolic Extract to Rice Crusts and Chemical Pesticide (Blitinute) in Inhibition of Fungi Isolate from Rice Seeds

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Abstract

Objective: The major goal regarding the presented study is to evaluate the impact of the Rice extract from Rice Crusts and chemical pesticide (Blitinute) on the growth of pathogenic fungi.

Method: Rice seed samples were collected directly from farmers field for isolating significant pathogenic fungi related to the seeds. Anti-fungal activity assay of the crude, Phenolic extract and chemical pesticide were determined by agar plating-method against some pathogenic fungi.

Result: obtained results showed the effect concentration of rice seed extract on growth of fungi especially phenolic extract had wide spectrum antifungal activity than crude extract. when the use of chemical pesticide (Blitinute) at a concentration of 0.3% with reaching rate of inhibition to 100% to *Fusarium graminerum* , *Penicillium viridicatum*, while (88.8) the same concentration for the *Aspergillus fumigates*. In another than the use Integrations between two extract and chemical pesticide (Blitinute) to high effect against test fungi than one extract and chemical pesticide.

Conclusion: This study demonstrates that the presence of three pathogenic fungi (*Aspergillus fumigatus* , *Fusarium graminerium*, *Penicillium viridicatum*) related to the rice grains have been controlled at various concentrations of two extract (crude & phenolic) and chemical pesticide totally inhibited radial growth regarding all test fungi .

Key Words: *Rise Crust, Crude & phenolic extract, Chemical pesticide, pathogenic fungi.*

Introduction

Continuously plants have been endangered through various pathogenic micro-organisms existing in their environments. Globally, phytopathogenic fungi is specified the major beneficent pest with regard to the farming harvests [1]. A Considerable amount of the agricultural products globally and in the country are not suitable for human's consumption, also they are not healthy because grains are polluted with mycotoxins, created via *Penicillium, Fusarium, and Aspergillus*. The reports have indicated that over three-hundred 300 fungal metabolites have high toxicity towards humans as well as animals, and 25% of the cereals in the world have been contaminated with certain mycotoxins [2].

The diseases produced from fungi form cause a significant loss of many economic crops in the world. The largest effect of fungi with regard to the decrease in efficiency of crops or post-harvest losses and leads to a huge loss to mankind [3].

Rice can be specified as a food of high significance that result in approximately 35% of the industrial costs, approximately 70% of the average calorie consumptions in addition to 93% of total produced food [4]. Worldwide, Rice (*Oryza sativa*) is a major cereal harvest. With regard to India, rice occupy the first in area of approximately 42. 24 million hectares and produce approximately 82 million tons [5]. Many studies on rice grain spotting as well as on its control were achieved home and abroad, yet the statistics regarding storages rice grain contain mycoflora as well as its control are not enough [6].

Plants are producing many types secondary metabolites regarding the low-molecular-mass which are typically isn't needed for basic metabolic procedures related to plants. A lot of secondary metabolites regarding these plants are recognized as allelochemicals which enhanced the protection against other plant competitions, insect/animal predation or microbial adhesion. Allelochemicals might be of high importance in the biological control regarding phytopathogens and weeds [7]. A lot of plant products and plants have anti-microbials against plant pathogenic fungi [8]. Control seed-borne fungal diseases and inhibiting the biodeterioration related to the grains with using use seed treatment is cost-effective and harmless approach [9]. Reports indicates that extracts of many plants were showing anti-bacterial, anti-fungal as well as insecticidal properties under laboratory trails. Appear plant metabolites in addition to the plant-based pesticides are considered as more effective alternatives since they have minimum environmental impact and dangers to the humans in a way similar to the synthetic pesticides [10]. Earlier, a lot of plant extracts which have source of bio-pesticide because contain substances inhibit the growth of plant pathogens and reduced the risk to environment and health of human. The presence of antifungal compounds in higher plants has long been recognized as important agents for the control of certain plant diseases [3]. Recently, many researchers in the world show interest in the application of plant product as bio- pesticide [11].

Aim of Objective:-

The present work aim was to evaluate the effect of Rice extract from Rice Crusts and chemical pesticide (Blitinute) on the growth of pathogenic fungi.

Methodology

Isolation of fungi from rice seeds:

Isolate the main pathogenic fungi from seed was collected rice seed samples directly from farmer's field. During the seventh day of incubation, examine the mycoflora seed samples with use microscopes. Identification 6 fungi based on growth characteristics, spore mycelial morphology, in addition to other features with applying standard guides.

The three species of *Aspergillus* and one species of each *Fusarium graminerum, Penicillium viridicatum, Cladosporium* spp were subculture using Potato Dextrose Agar (PDA) medium.

Preparation of Rice Seed Extracts:

- **Crude Extract**

Weight 100g of Crusts rice powder and put in a 1000 ml glass flask and add 200 ml of ethyl alcohol and leave for 48 hours, taking into reflection the shaker Periodically from time to time, the filter was filtered using filter paper (Whatman No.4) and by vacuum, The extract was concentrated using (Rotary vacuum Evaporator) at a temperature of 45ºC, then the weight of the concentrated extract [12], the purpose of the biological activity testing to the raw extract in the test fungi, take 0.3g of dry extract and dissolve in 1ml of ethyl alcohol 99% and then complete the volume to 10ml by distilled water. Concentration is 3%. For control, it is 1ml of ethyl alcohol and full size with distilled water to 10 ml.

- **Phenolic Extract**

The method was followed by [13] to extract the phenolic compounds from the Crusts rice, take 20g dry weight of the crusted crust and put in a 500ml glass flask, and add 400 ml of 2% HCl. The phenolic compounds were extracted using a water bath at 100 °C for one hour. After the end extraction process, the solution was cooled and filtered with the filter paper type Whattman No.1 and put filter in the separating funnel and added an equal volume of n-propanol, and then added to the amount from NaCl to reach the state of saturation, then formed two layers, isolated the upper layer containing phenolic compounds, and focused on rotary evaporator, then placed in the oven at 40°C to dry, and reduced in a refrigerator for use, For the purpose of testing the biological activity of the phenolic extract in the test fungi, take 6g of phenolic extract and dissolve in 8ml of ethyl alcohol 90% and then complete the volume to 25ml by distilled water. Concentration is 24%. For control, it is 8ml of ethyl alcohol and full size with distilled water to 25 ml.

Anti-fungal activity assay:

The preparation of media PDA in two flask the volume each flask about 500ml with the 400 media/ flask, then sterilization with autoclave, after sterilization leave to cool, before solidification of the medium to content pour 18 plate, in average 20ml/media, 9 plate content extract to 1ml each plate, while another 9 plate content solution ethyl alcohol and distilled water (control treatment) at 1ml/plate, after solidification of the medium to culture 5mm disc of 7-day-old culture of

test fungi have been inoculated, there are 3 replicates have been maintained for all extracts. Plates have been incubated at a temperature of 22±1 Celsius for 7 days. after the percentage inhibition regarding mycelia growth has been estimated with the use of formula based on [14].

% inhibition = dc – dt x 100/dc

where:

dc = average increase in mycelia growth in control.

dt = average increase in mycelia growth in plate content extraction.

Result

Isolation and Identification:

Show fungi on seed borne the percent incidence associated with rice seed, the data revealed that the *Aspergillus fumigates, Fusarium graminerum* , *Penicillium viridicatum* with the high frequencies, the percent of frequencies include (93,90,89)% respectively. While other include *Aspergillus terrens*, *Aspergillus niger*, *Cladosporium* spp the percent (77, 63, 58)% respectively .

The results of Reagent use to detect crude extract of rice crust, showed that the alkaloids reagent includes (Drakandroff) negative, while positive of (Mayer, Tannic). Phenolic Reagent include (Lead acetate) positive, while negative of Potassium hydroxide. Turpinnes Reagent include (Foam & Mercury chloride) negative.

Effect concentration of rice seed extract and chemical pesticide (Blitinute) in growth inhibition test fungi:

Figure (1) indicates that decrease colony diameters by gradient concentration, with a 5% concentration being the most effective among concentrations (46.55, 52.22, 51.33) for fungi *Aspergillus fumigates, Fusarium graminerum* , *Penicillium viridicatum* respectively, compared to control treatments.

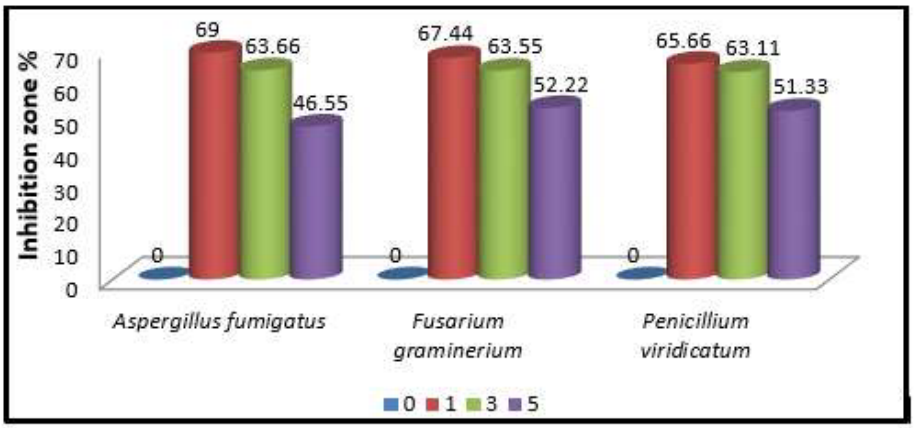

Figure (1): Effect crude extract of Rice Seed to different concentrations (0,1,3,5)% on growth inhibition of test fungi

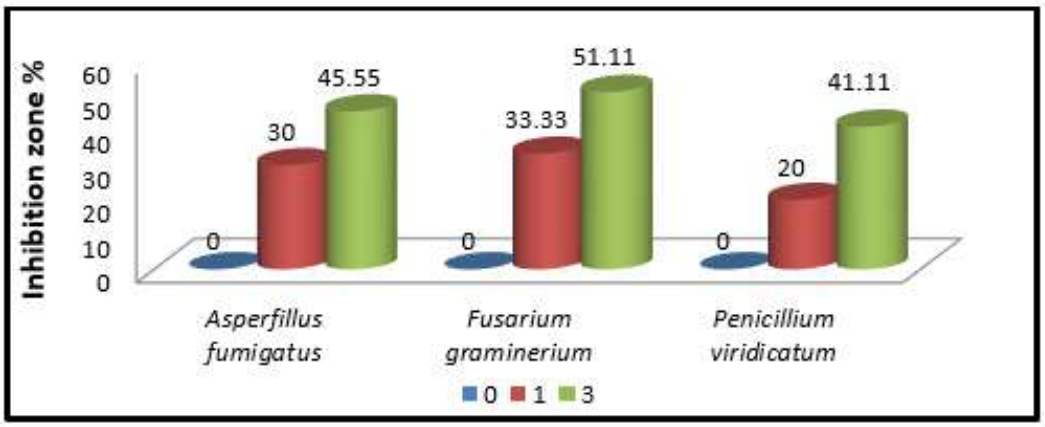

Figure (2): Effect phenolic extract of Rice Seed to different concentrations (0,1,3)% on growth inhibition of test fungi

Figure (2) show that the phenolic extract of rice seed give high efficiency at 3% concentration as decrease the growth rates of the studied fungi to (45.55, 51.11, 41.11) for fungi *Aspergillus fumigates, Fusarium graminerum* , *Penicillium viridicatum* respectively.

The results are shown in figure (3) to decrease the growth rates of fungi and especially *Fusarium graminerum* , *Penicillium viridicatum* when the use of chemical pesticide (Blitinute) at a concentration of 0.3% with reaching rate of inhibition to 100% while (88.8) the same concentration for the *Aspergillus fumigates*.

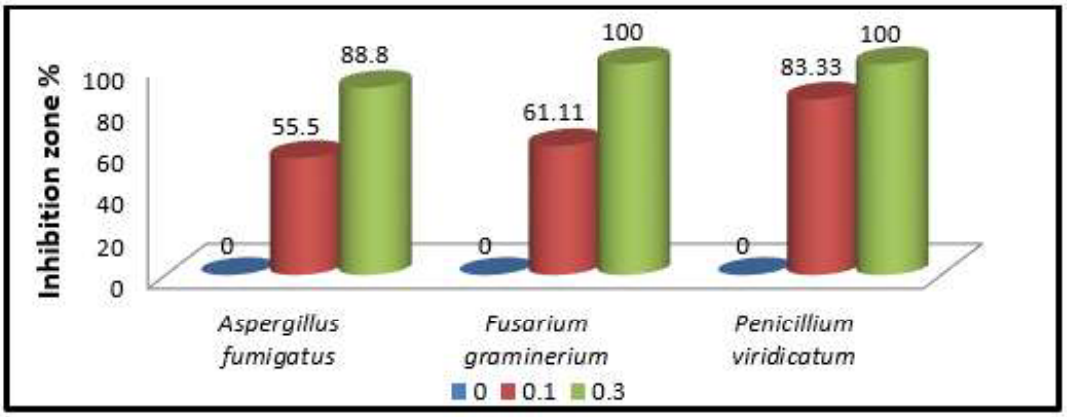

Figure (3): Effect chemical pesticide (Blitinute) to different concentrations (0,0.1,0.3)% on growth inhibition of test fungi

Integration activity between Rice crusts extract and chemical pesticide (Blitinute) in inhibition of test fungi

The result in table (1) shown that the use integrations between crude extract at concentration 5% and chemical pesticide at concentration 0.3 to reduce contamination of seed by fungi *Aspergillus fumigatus* , *Fusarium graminerium*, *Penicillium viridicatum* to (89,94,80) respectively.

Table (1): Integrations use between Active Concentration of crude extract and chemical pesticide (Blitinute) against test fungi

Fungi	**Active Concentration**	**Percentage %**
Aspergillus fumigatus	5% crude extract + 0.3 chemical pesticide (Blitinute)	89
Fusarium graminerium	5% crude extract + 0.3 chemical pesticide (Blitinute)	94
Penicillium viridicatum	5% crude extract + 0.3 chemical pesticide (Blitinute)	80

While use integrations between phenolic extract at concentration 3% and chemical pesticide at concentration 0.3 to reduce contamination of seed with the same fungi to (87,99,81) respectively, as show in table (2).

Table (2): Integrations use between Active Concentration of phenolic extract and chemical pesticide (Blitinute) against test fungi

Fungi	**Active Concentration**	**Percentage %**
Aspergillus fumigatus	3% phenolic extract + 0.3 chemical pesticide (Blitinute)	87
Fusarium graminerium	3% phenolic extract + 0.3 chemical pesticide (Blitinute)	99
Penicillium viridicatum	3% phenolic extract + 0.3 chemical pesticide (Blitinute)	81

The result in table (3): show efficiency use two extract (crude & phenolic) at concentration 5%& 3% respectively and chemical pesticide in concentration 0.3 to reduce seed contamination with fungi *Aspergillus fumigates*, *Fusarium graminerium*, *Penicillium viridicatum* to (94, 100, 90) respectively.

Table (3): Integrations use between active concentration of crude, phenolic extract and chemical pesticide (Blitinute) against test fungi

Fungi	Active Concentration	Percentage %
Aspergillus fumigatus	0.3 chemical pesticide (Blitinute) + 5% crude extract + 3% phenolic extract	94
	Control	0
Fusarium graminerium	0.3 chemical pesticide (Blitinute) + 5% crude extract + 3% phenolic extract	100
	Control	0
Penicillium viridicatum	0.3 chemical pesticide (Blitinute) + 5% crude extract + 3% phenolic extract	90
	Control	0

Discussion

Botanist can be specified as rich storehouse related to the natural chemicals which might be exploited to be used as pesticides. Reports indicates the overall quantity regarding plant chemicals might be over 4,000,000 and of these 10,000 are to be create secondary metabolites which are of high importance in the plant's protective them. [15]. Previous researches indicate that anti-microbial activity includes anti-fungal properties with the use of diverse classes regarding the extracts [16]. The reports indicated creating considerable decrease in nutritious quality and seed quality because of the association to fungi ranges [17]. Other studies have proven the effective role of medicinal plants in eliminating various pathogen [18,19,20]. Not been described a lot of species regarding advanced plants have extremely less studied for chemical or biologically active components and novel sources of commercially valuable pesticides [10]. Various studies indicate a major alternative for better alternatives plant metabolites and plant based pesticides as recognized to have reduced environmental impact and hazard to the consumers when compared to the synthetic pesticides [21]. This study is consistent with other studies in the inhibition of pathogen using probiotics in the treatment of multiple microorganism resistant to antibiotics [22,23]. The attributed to destruction regarding the occurrence of seed borne fungi which might have killed embryo of seeds because of the ability of extracts for increasing seed germination as well as seedling development. Such results are in accordance with the results of [24] who indicated that leaf extracts of *C. viscosum* have increased the growth of seeds as well as improving the development regarding rice seeds. Plant extracts are considered to be bio-pesticide control agent which are of high importance due to 2 certain reasons. The first on is it can be safely used for individuals as well as environmental accumulation. The second reason is its capability for controlling pathogens as well as inhibiting pathogens from the emerging resistance to fungicide [25]. The main step is application of the bio-pesticides for using natural products from the plants as measure for controlling and inhibiting plant diseases. This result consistent with another studies[26]. Such results are in accordance with [27] for showing the impacts of 2 biocides ("Bacillin and Floramyle") on inhibition the fungus growth (*Aspergillus flavus* and *Aspergillus niger*) for rice seed contamination.

Conclusion

The main step of this study demonstrates in the

development of pesticides based on plant that are environmentally approachable for the controlling fungi on seed-borne and the improvement of commercial preparations of plants, and revealed the presence of three pathogenic fungi (*Aspergillus fumigatus, Fusarium graminerium, Penicillium viridicatum*) on rice grains were controlled in different concentrations, the two extracts (crude and phenolic) and chemical pesticides completely inhibit the radial growth of all test fungi.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: The authors declare no conflict of interest.

Ethical Clearance: All experimental protocols were approved under the Al-Furat Al-Awsat Technical University, Kufa Technical Institute/Iraq and all experiments were carried out in accordance with approved guidelines.

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