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Effect of Some Algal Filtrates and Chemical Inducers on Root-Rot Incidence of faba Bean

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Abstract

Some algal filtrates namely Sargassum, Fucus and Nostoc spp., and chemical inducers namely Dipotassium Phosphate (K_2HPo_4), Salicylic acid and Ascorbic acid to investigate their effect on the control of faba bean root-rot caused by *Rhizocptina solani*, *Fusarium solani* and *Fusarium oxysporum* in the laboratory and green house using the faba bean (Giza3 Mohassan). Results of *in vitro* studies showed that algal filtrates or chemical inducers significantly inhibited the mycelial growth of the pathogen, when compared to the untreated control. Under greenhouse conditions, all isolated fungi proved to be pathogenic and caused pre- and post emergence damping-off. Considerable increases in activity of oxidative reductive enzymes (peroxidase and polyphenol oxidase) and chitinase enzyme were recorded in plants grown from treated faba bean seeds.

Keywords: Algal filtrates; Chemical inducers; Root-rot; Chitinase; Peroxidase; Polyphenol oxidase

Introduction

Faba bean (*Vicia faba* L.) is a legume crop with high nutritional value. It contains about 18.5 and 37.8 % protein [1]. Faba bean plants are reacted with many fungal pathogens which cause considerable yield losses [2]. Fungi belonging to various genera were isolated from infected plants. *Rhizoctonia solani* was the most pathogen causing root-rot diseases [3-5] isolated *Rhizoctonia solani* and *Fusarium oxysporum* from wilted and rotten roots of faba bean in different parts of the world. Abdel-Kader [6] several root rot and wilt pathogen such as *Rhizoctonia solani*, *Fusarium oxysporum* and *Macrophomina phaseolina* are reported to attack faba bean roots and stem base causing serious losses in seed germination and plant stand as well. However, algal filtrates have been record as active inhibitors to the *in vitro* fungal growth [7,8] found that all the tested algal filtrates except *Anabaena* decreases the mycelial growth of *S. Sclerotiorum* as compared with control. It may be possible to utilize a scheme to inducible plant defenses which provides protection against a broad spectrum of diseases causing organisms. Among synthetic inducer salicylic acid (S.A.) and hydrogen peroxides (H_2O_2) have been found to be active as antimicrobial agents in various trials as diseases resistance inducers. Theses have been reported for inducing resistance agents several plant pathogens such as *F. oxysporum* in tomato [9]. El-ghanam [10] reported that, the percentage of diseases severity of fruits rot decreased by spraying strawberry fruits with all different algae in field and laboratory.

Abdel-Monaim [9] found that S.A. follow by H_2O_2 recorded the lowest reduction of growth in all tested fungi. Several investigators studied the effectiveness of these chemical inducers on root rot disease [11]. The treatment sensitizes the plant to respond rapidly after treatment. These responses include phytoalexin an accumulation, phenol, lignifications and activation of peroxidase, polyphenol oxidase and chitinase.

Therefore, the objectives of the present study were to study the effect of algal filtrates and chemical inducer on root rot diseases of faba bean, in order to increase the number of survival plants.

Material and Methods

The present study dealt with the management of root rot disease of faba bean under laboratory experiments and green house conditions.

Source of inocula

The concerned disease caused by *Rhizocptina solani*, *Fusarium solani* and *Fusarium oxysporum* pathogenic fungi were obtained from infected roots of faba bean collected from different locations throughout Egypt. The used isolates were purified and identified following the methods adapted by Sneh et al. [12]. Different experiments were carried out in a randomized complete plot design with three replicates for each treatment using the cv.Qiz93 Mohassan were obtained from the Field Crop Institute, Agricultural Research Center, Ministry of Agriculture, Egypt.

Laboratory experiments

Algal materials and filtrate: two seaweeds were collected from Suez Canal (Egypt) at Depressor during June 2014. The algae were collected and transported to the laboratory within 8 hours in a tank with sea water. The algal species were identified based on the schemes reported in the literature [13-20].

Algal cultures were grown in enriched artificial or natural seawater. Artificial seawater was based on the recipe by Harrison et al. [21] as modified by Thompson et al. [22]. Natural seawater was treated with activated charcoal (to remove organic compounds), gravity filtrated through a Whatman 1 filter paper, re filtrated through 0.8 µm Millipore filter autoclaved and then enriched with ES levels of nutrients trace metals and vitamins [21]. Cultures were exposed to continuous light (provided by Vita-lite fluorescent tubes). At 19 °C and 35µ mol photo $ns^{-2}S^{-1}$. Two weeks later, the developed algal growth was filtrated under aseptic conditions to obtain culture filtrates. *Nostoc sp.* was kindly supplied by Botany and Microbiology Department-Faculty of science (for girls) Al-Azhar University Cairo- Egypt.

Nostoc sp. were diluted with sterilized distilled water to make 1/10 dilution series. Ten serial dilutions were then carried out with sterilized distilled water one ml of 10^{-2} to 10^{-6} dilutions were poured on the surface of the agar medium and spread with glass spatula, then incubated in inverted position at room temperature for 3 weeks. Isolation was carried out using dissociating microscopic and *Nostoc* colony was picked out using inoculation needle for propagation, purification and identification. Slant agar refrigerated *Nostoc* (at 5 °C) was Exposed to light (500 Lux) for 2 days then inoculated to a liquid medium. *Nostoc* grew in 500 ml conical flasks containing 100 ml sterilized BGII medium ($MgSO_4 7H_2O$, $K_2HPO_4 3H_2O$, $Cacl_2\ 2H_2O$, Na_2CO_3, Citric acid) and incubated under initiation (3000 Lux) at temperature 28-32 °C. Two weeks later, the developed *Nostoc* growth was filtrated under aseptic conditions to obtain culture filtrates.

Effect of some algal filtrates on mycelial growth of pathogenic fungi

The crude filtrate of each alga, prepared as mentioned before was individually mixed with PDA medium at the rate of 1 ml/gmL media at 45 °C and poured into plates [23]. Plates centers were incubated at 20 °C for 5 days. Algal filtrate free PDA plates were used as control.

The effect of some chemical inducers on mycelial growth of pathogenic fungi *in vitro*

The effect of different concentrations of three chemical inducers, K_2HPO_4 (10, 25, and 50 mM), Salicylic acid (1, 2, 10 mM) and Ascorbic acid (1, 2, 10 mM) were added to PDA medium and then poured in Petri dishes (Dimensions: (DxH) 94mm x 16mm) and inoculated in center with plates were incubated at 27 °C for 7 days. The diameter of pathogen radial was calculated as mentioned previously.

Green House Experiment

Effect of soil treatment with some algal filtrates on soil treatment with some algal filtrates on damping-off of faba bean: -

Crude algal filtrates are individually added to the soil infested with *R. solani*, *F. solani* and *F. oxysporum* isolates (30 ml/ Kg soil) at the rate of 90 ml/pot (20-cm-diam). Seeds of faba bean were sown at rate of faba bean were sown at the rate of faba bean were sown at the rate of 10 seeds/pot (Giza 3 Mohassan). Soil treated with water only was served as control. Three replicates were used for each treatment.

Diseases assessment

Percentage of pre- and post emergence damping-off as well as healthy survival plants in each treatment were determined15 and 30 days after sowing respectively using the formula according to [24]

$$Pre-emergence\ (\%) = \frac{Number\ of\ non\ germinated\ seeds \times 100}{Total\ number\ of\ sown\ seeds}$$

$$Post-emergence\ (\%) = \frac{Number\ of\ dead\ seedling \times 100}{Total\ number\ of\ sown\ seeds}$$

$$Survival\ plant\ (\%) = \frac{Number\ of\ survival\ plant \times 100}{Total\ number\ of\ sown\ seeds}$$

The plant height of faba bean plants and disease severity were also recorded after 35 days from sowing.

Effect of soaking faba bean seeds in some chemical inducers agents on controlling root rot under green house conditions

Faba bean seeds were soaked in the solution of each tested chemical for 2.5 hrs [25] before planting. Each particular treatment consisted of one concentration K_2HPO_4 (50 mM), Salicylicacid10 mM and Ascorbic acid 10 mM. The welted seeds were spread in the thin layer and left about 24 hours, then sown in the infested potted soil with the virulent *R. solani*, *F. solani* and *F. oxysporum* isolates. Seeds of Giza 3 Mohassan were soaked in tap water and were sown in pots to serve as control. Three pots for each treatment were used as replicates. Pre-post emergence root rot and survival plant incidence were recorded after 15 and 30 days of sowing. The height of faba bean plants and disease severity were also recorded after 35 days from sowing.

Fresh samples were taken from plants grown from previously treated and untreated faba bean seeds and extracts were used for assaying biochemical change associated with tested treatments of algal and chemical inducers on the activities of peroxidase enzyme [26] and polyphenol oxidase enzyme [27] and chitinase enzyme [28] were determined.

Statistical analysis

The obtained data were statistically treated by analysis of variance (ANOVA) using the fisher L.S.D. method. Means were separated by Fisher's protected least significant differences [29].

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Results

All the tested algal filtrates significantly reduced the mycelia growth of *R. solani*, *F. solani* and *F. oxysporum* as compared with the control Table 1 filtrates of *Sargassum*, *Fucus* and *Nostoc* spp. completely inhibited the mycelia growth of the fungi.

Effect of some chemical inducers on mycelia growth of fungi under laboratory condition

The effect of the tested chemicals on mycelia growth of *R. solani*, *F. solani* and *F. oxysporum* are shown in Table 2. Data indicate that low concentrations of Salicylic acid (S.A.), Ascorbic acid (A.A.) and K_2HPO_4 slightly reduced the linear growth of fungi. The highest reduction in the linear growth was achieved when Salicylic acid and Ascorbic acid followed by K_2HPO_4 (Dipotassium phosphate).

Effect of soaking seeds in some algal filtrates on root incidence under green house conditions

Data in Table 3 indicate that the tested algae significantly reduced pre and post emergence damping-off caused by of *R. solani*, *F. solani* and *F. oxysporum* compared to the control. However, *Nostoc spp*. was the most effective tested algal filtrates on decreasing the percentage of pre- and post emergence root-rot followed by *Fucus* and *sargassum*. However all treatment significantly reduced the disease severity root-rot symptoms caused by *R. solani*, *F. solani* and *F. oxysporum*.

Effect of the tested chemical inducers on root rot incidence under green house condition

Data in Table 4 show significant effects of salicylic acid, Ascorbic acid and Dipotassium Phosphate (K_2HPO_4) on reducing the percentages of pre- and post emergence damping off caused by *R. solani*, *F. solani* and *F. oxysporum* compared to the control. However, Salicylic acid was the most effective tested chemical inducers on decreasing the percentage of pre- and post emergence root rot. However, all treatments significantly reduced the disease severity of root rot symptoms caused by *R. solani*, *F. solani* and *F. oxysporum.*

Effect of soaking faba bean seeds in some algal filtrates and solution of some chemical inducers on the biochemical changes of faba bean plant in soil infected with *R. solani, F. solani* and *F. oxysporum*.

Effect on peroxidase and polyphenol oxidase activity

Data presented in Table 5 & 6 indicate that faba bean plants grown from seeds soaked in some algal filtrates and solutions of some chemical inducers resulted in an increase of peroxidase and polyphenol oxidase activity compared to the untreated control.

Fucus and *Nostoc* gave the highest increase in peroxidase activity followed by Salicylic acid. Meanwhile Ascorbic acid gave the least activity compared to control (check) treatment. Furthermore, *Nostoc* gave the highest increasing in polyphenol oxidase activity followed by *Fucus* and Salicylic acid followed by K_2HPO_4 respectively. Meanwhile Ascorbic acid gave the lowest value compared to control (check) treatment.

Chitinase enzyme activity

Data in Table 7 recorded that treatment of faba bean seeds with some algal filtrates and chemical inducers resulted in increase chitinase activity compared to the untreated control (check). *Nostoc* gave the highest increasing followed by *Fucus* while Ascorbic acid gave the least compared to other treatments.

Discussion

Faba bean (*Vicia faba* L.) is legume crop with high nutritional value. Soil borne diseases including root-rot cause considerable yield losses. The surveys showed differences in the frequency of the isolated fungi. Similar results were early reported by El-sayed [1], [2,5,30,31]. Testing the inhibitory activities of algal filtrates on mycelial growth of *R. solani*, *F. solani* and *F. oxysporum* demonstrated that *Fucus* and *Nostoc* spp was the most effective antifungal agent against *R. solani*, *F. solani* and *F. oxysporum*. This activity might indicate the ability of algae to produce bioactive secondary compounds, secreted into the surrounding medium. These bioactive ingredients seem to hinder growth of the isolates of the tested fungi (*Fucus* and *Nostoc spp.*) as they have the ability to produce variety of lethal toxins [32]. These results are in agreement with Caccamese et al. [33] who reported that cyanobacteria are probably best known for production of toxins by certain species that live in both fresh and salt water. These results are somewhat similar to those obtained by Biondi et al. [23] who mentioned that adding culture filtrate of *Nostoc* strain to soil resulted in complete inhibition of fungi

The effectiveness of soaking seed in algae filtrates might be due to the absorption of active substances which prevented the infection and disease development. As for soil treatment Biondi et al. [23] stated that the efficacy of irrigated soil with culture filtrates may be due to the capability of antifungal like substances to penetrate into the fungal cell, consequently causing alterations in fungal metabolism. Furthermore, induced systemic resistance of plants against pathogens in wide spread phenomenon that has bean investigated with respect to the underling signaling pathways as well as its potential use in plant protection [34]. The tested chemical inducers might stimulate some defense mechanisms such as phenolic compounds, oxidative enzymes and some metabolites.

The present work demonstrated that application of algae and chemical inducer resulted in plant health. These treatments caused a significant reduction in root rot incidence of faba bean. In addition, the activity of peroxidase and polyphenol oxidase and chitinase enzymes were obviously higher in plants grown from treated seed compared to the untreated. In general, salicylic acid, *Nostoc* and *Fucus* were the most effective for stimulating these defense mechanisms. The present results are in agreement with those recorded by Ibrahim [35]. It may be concluded that application of algae filtrate is considered an applicable, safa and cost-effective method for controlling such soil –borne disease.

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