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

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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 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

Abstract

Some algal filtrates namely Sargassum, Fucus and Nostoc spp., and chemical inducers namely Dipotassium Phosphate (K$_2$HPO$_4$), Salicylic acid and Ascorbic acid to investigate their effect on the control of faba bean root-rot caused by Rhizoctina 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.

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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$_2$O$_2$) 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$_2$O$_2$ 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 Rhizoctina 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 seawater. 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 filtered 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 35 µmol photo ns-2. 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 BG11 medium (MgSO_4·7H_2O, K_HPO_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/gm Mitsubishi 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_HPO_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]

\[
\text{Pre -- emergence} \% = \frac{\text{Number of non germinated seeds} \times 100}{\text{Total number of sown seeds}}
\]

\[
\text{Post -- emergence} \% = \frac{\text{Number of dead seeding} \times 100}{\text{Total number of sown seeds}}
\]

\[
\text{Survival plant} \% = \frac{\text{Number of survival plant} \times 100}{\text{Total number of sown seeds}}
\]

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_HPO_4 (50 mM), Salicylic acid10 mM and Ascorbic acid 10 mM. The wetted 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].
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$_2$HPO$_4$ 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$_2$HPO$_4$ (Dipotassium phosphate).

Effect of soaking seeds in some algal filtrates on root incidence under greenhouse condition

Data in Table 3 indicate that the tested alga significantly reduced pre and post emergence damping-off caused by *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 greenhouse condition

Data in Table 4 show significant effects of salicylic acid, Ascorbic acid and Dipotassium Phosphate (K$_2$HPO$_4$) 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$_2$HPO$_4$ 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 in 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 been 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, safe and cost-effective method for controlling such soil-borne disease.
How to cite this article: EL-syed SA, Mousa AM. Production, Effect of Some Algal Filtrates and Chemical Inducers on Root-Rot Incidence of faba Bean. Agri Res Tech Open Access J. 2015;1(1): 555552.

References

1. El-sayed F, Nakoul H, Williams P (1982) Distribution of protein content in the collection of faba bean (Vicia Faba L.) FABIS 5: 37.
2. Mahmoud NM (1996) Studies on chocolate spot diseases of broad bean and Loss occurrence. Ph D. Thesis Fac Agric Minufiya Univ pp. 133.
3. El- Morsy GA, Abou-zeid NM, Hassanein AM (1997) Identification of Fusarium wilt caused by Fusarium oxysporum and pathogen variability in faba bean, Lentil and chickpea crops in Egypt. Egyptian J Agriculture Research 75: 551-564.
4. Akem C, Bellar M (1999) Survey of Faba bean (Vicia faba L) diseases in the main faba bean-growing regions of Syria. Arab J Plant Protection 17: 113-116.
5. Hugar MPAA (2004) Effect of adding some biocontrol agents on some target microorganisms in root diseases in infecting soybean and broad bean plants. M. Sc. Thesis Fac. Agric, Moshtoher, Benha Branch, Zagazig University.
6. Abdel-Kader MM, El-Mougy NS, Lashim SM (2011) Essential oils and trichoderma harzianum as an integrated control measure against faba bean root rot pathogens. J plant Prot Res 51(3): 306-313.
7. Zacco GZ, DEcano MS, Galvagno M (2006) Action of cell free extracts and Extracellular products of Nostoc muscorum on growth of S. sclerotiorum. Phyton 47(1-2): 43-46.
8. Soliman NK, Mansour MS, Hilal AA, Redaw MSS (2009) Performance of algal filtrates and biocides for the control of sclerotinia sclerotiorum causing damping-off of fennel. J Phytopathology 37(2): 45-57.
9. Abdel-Monaim MF (2013) Improvement of Biocontrol of damping-off and root-rot/wilt of faba bean by saliculic acid and hydrogen peroxide. Microbiology 41(1): 47-55.
10. El-ghanam AA, Farfou SA, Ragab SS (2015) Bio-suppression of strawberry fruit root diseases caused by Botrytis cinerea. J plant pathol Microbial3: 2-7.
11. Segarra G, Jouregui O, Casanova E, Trillas I (2006) Simultaneous quantitative Lc-ESI-MS/MS analyses of salicylic acid and jasmonic acid in crude extracts of cucumis sativus under biotic stress. Phytochemistry 67(4): 395-401.
12. Sneh B, Burpee L, Ogashi A (1992) Identification of Rhizoctonia species. APS. Press. USA, pp. 133.
13. Nasr AH, Akem AA (1949) Ecological studies of some marine algae from Alexandria. Hydrobiology 1(1): 251-281.
14. Smith GM (1944) Marine algae of the Monterey Peninsula. Reprinted from Alexandria. Hydrobiologia 1(1): 251-281.
15. Levrin T (1946) A list of marine algae from Australia and Tasmania. Acta Horti Gothenburg 16: 215-231.
16. Bouck GB (1965) Fine structure and organelle associations in brown algae. J Cell Biol 26(2): 523-537.
17. Scagel F (1966) The phaeophyceae in perspective oceanogr. Mar Biol Ann Rev 4: 123-194.
18. Bold HC (1978) “Introduction to the Algae” Structure and Reproduction Prentice Hall, Inc., New-Jersey 07632.
19. Aleem AA (1993) The Marine Algae of Alexandria. Egyptian Books House, Egypt.
20. Coppejans E, Leliaert F, Dargent O, Gunasekara R, Clerck O (2009) Sri lankan Seaweeds. Methodologies and Field Guide to the Dominant Species. University of Ruhuna, Dept of Botany, Matura, Sri lanka, pp. 1-265.
21. Harrison PJ, Waters RR, Taylor FJR (1980) Abroad spectrum artificial seawater medium for coastal and open ocean phytoplankton. J Phycol 16(1): 28-35.
22. Thompson PA, Harrison PJ, Parslow JS (1991) Influence of irradiance on cell volume and carbon quota for ten species of marine phytoplankton. J Phycol 27(3): 351-360.
23. Biondi N, Piccardi R, Margheri MC, Redolfi L, Smith GD, et al. (2004) Evaluation of Nostoc strain ATCC53789 as a potential source of natural pesticides. Appl Environ Microbiol 70(6): 3313-3320.
24. El-Helaly AF, Elaroos H, Assawah MW, Abol-wafa MT (1970) Studies on damping-off and root-roots of bean in UAR (Egypt) J Phytopathol 2: 41-57.
25. Khaleefa MMA, Draiz Et mad El, Ibrahim MM (2007) Charcoal rot of sunflower in Egypt: performance of some various control measures on diseases incidence and seed yield production. Egypt J Appl Sci 22: 315-330.
26. Allam AI, Hollis JP (1972) Sulfide inhibition of oxidase in rice roots. Phytopathology 62: 634-639.
27. Snell FD, Snell CT (1953) Colorimetric Methods of analysis, Including and Turbidmetric and nephelometric Methods. Dvan Nostrand company Inc. Toronto New York, London, USA, III 606.
28. Tuzun S, Rao MN, Vogeli U, Schardi CL, An Kue J (1989) Induce systemic resistance to blue mould: early induction and accumulation of B-1,3 glucanase, chitinase and other pathogenesis-related protein in immunized tobacco. Phyto pathology 79: 979-983
29. Gomez KA, Gomez AA (1984) Statistical Procedures for Agricultural Research. (2nd edn.), A wiley Inter. Science publication. John wily& sons. Inc. New York, USA, pp. 680.
30. Abdel-Kader MM, El-Mougy NS (2013) Bio agents and commercial algae products as integrated biocide treatments for controlling root rot diseases of some vegetables under protected cultivation system. J of Marine biology 2013: 1-10.
31. Metwaly MMM (2004) Resistance induction against diseases of faba bean crop. Ph D. Thesis plant pathology Dept. Fac Agric Suez Canal Univ. Egypt.
32. Sunakk A, Sihoonen LM, Vuorela P, Sivonen K, Lehtimaki JM, et al. (2005) Benthic cyanobacteria from the Baltic sea cytotoxic Anabaena,
Nodularia and Nostoc strains and an apoptosis-inducing Phormidium strain. Environ Toxicol 20(3): 285-292.

33. Caccamese S, Azzanlina RA, Furnari G, Grasso S (1981) Antimicrobial and antiviral activities of some marine algae from Eastern Sicily. Bot Mar 24(7): 365-367.

34. Mona MMR, Saber MM, El-Morsy SA, Abeer RM, Abd El-Aziz (2009) Induction of systemic resistance against root-rot of basil using some chemical inducers Egypt. J Phytopathol 37(1): 59-70.

35. Ibrahim MMA (2006) Studies of charcoal rot disease cause by Macrophomina phaseolina sunflower and its control. Ph. D. thesis, Faculty Agric., Ain-Shams Univ. pp. 148.