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Unravelling *Aspergillus*-*Fusarium* co-culture impact on biometry of *Fusarium* basal rot in onion

Abdelrahem M. M. M.[a], Abo-Dahab N. F.[a], Hassane A. M. A.[a*], Abouelela M. E.[b]

[a]*Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Assiut Branch, Assiut 71524, Egypt*
[b]*Department of Pharmacognosy, Faculty of Pharmacy (Boys), Al-Azhar University, Cairo 11884, Egypt*

Abstract

Onion basal rot, a serious and persistent disease problem caused by *Fusarium proliferatum*, results in severe yield reductions, impacting both quantity and quality of onion worldwide. *Fusarium proliferatum* penetrates roots leading to decay and wilting of the onion plant. It is important to manage this disease to minimize its effects. Here, we investigated the bioactivity of co-culture extract of *Aspergillus ochraceus*-*F. proliferatum* (AF) against *F. proliferatum* responsible for *Fusarium* basal rot (FBR) in a greenhouse experiment. The results demonstrated that the AF co-culture extract treatment reduced the percentage of infected plants to 0% and significantly enhanced all growth parameters in infected onion plants. Moreover, the treatment with AF co-culture extract performed the highest values in infected onion plant with total pigments of 3.24 mg/g, carbohydrates of 48.95 mg/g, proteins of 105.23 mg/g, phenolics of 41.64 mg/g, and flavonoids of 8.03 mg/g compared with monocultures extracts and chemical fungicide treatments, and healthy control plant. This establishes that fungal co-cultures bioagents represent a promising prospect as substitutes to chemical fungicides beside ameliorating plant growth.
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Keywords: *Fusarium* basal rot, onion, co-culture, greenhouse, growth parameters.

*Corresponding author: Hassane A. M. A.,
E-mail address: abdallahhassane@azhar.edu.eg

1. Introduction

Fungi exist in a variety of habitats where soil, endophytic, epiphytic, and mycorrhizal fungi possess diverse of functions, and their primary and secondary metabolites offered a variety of biological activities and biotechnological applications (Mohamed *et al.*, 2021). More than 38% of the biologically active metabolites were derived from fungal sources (Higginbotham *et al.*, 2013). These metabolites encompass nutraceutical, medical, and biotechnological polysaccharides, lipids and fatty acids, and enzymes and peptides (Abdou *et al.*, 2024; Abdeen *et al.*, 2024; Al Mousa *et al.*, 2022a,b; Giavasis, 2014; Hassane *et al.*, 2024; Khalaf *et al.*, 2024; Mohamed *et al.*, 2022) as well as low molecular weight secondary products including mainly phenolic acids, alkaloids, saponins, flavonoids, and terpenoids (Pimentel *et al.*, 2011). Fungal secondary metabolites were proved to exhibit diverse antibacterial, antifungal, anticancer, antioxidant, and wound healing properties (Al Mousa *et al.*, 2022c; 2024a,b; Hassane *et al.*, 2022a,b). On the other hand, fungi display a substantial role in the production of different mycotoxins (Abo-Dahab *et al.*, 2016; Hassane *et al.*, 2017; 2018; Saber *et al.*, 2016). Culturing the microorganisms as mono-cultures in normal laboratory conditions predominatingly fail to unlock their ultimate metabolic diversity, due to absence of ecological stimuli indispensable for inducing biosynthesis gene clusters (Kwon *et al.*, 2019). Microbial co-culture represents a powerful tool for inducing metabolite biosynthesis and enhancing chemo-diversity. Combining ecological principles with modern genetic and biochemical techniques has enabled researchers to unlock a wealth of bioactive compounds that may lead to new therapeutic agents (Selegato and Castro-Gamboa, 2023). *Allium cepa* (onion) represent one of the most important commercial crops, widely grown in different countries around the world (Gebretsadik and Dechassa, 2018), and known for its valuable nutraceutical and medicinal properties (Omar *et al.*, 2020). Onion is extremely exposed to *Fusarium* basal rot (FBR), associated with different species of *Fusarium*, a major restriction to onion yields worldwide. Several agronomic approaches have been carried out to control *Fusarium* basal rot, comprising solarization, fungicides, soil fumigation, resistant cultivars, and long crop rotation (Cramer, 2000). However, fungicides have adverse effects on people, animals, and the environment which led to their limitation in many countries (Aktar *et al.*, 2009; Fan *et al.*, 2008). Thus, natural products have been investigated to control myco-pathogens to underscore the need for alternative natural, safe, and effective plant protection agents (Oppong-Danquah *et al.*, 2020), and to minimize the use of synthetic fungicides (Rongai *et al.*, 2015). The present study is an extended investigation of the interesting *Aspergillus*-*Fusarium* co-culture, profiled using HPLC and established *in vitro* promising anti-*Fusarium* potency (Abdelrahem *et al.*, 2023). Herein, assessment of *in situ Aspergillus*-*Fusarium* co-culture impact on onion-*Fusarium* basal rot plants parameters was conducted. Furthermore, these findings shed light for further study regarding bioassay-guided fractionation and commercial applications of these bioagents.

2. Materials and methods

2.1 Fungal strains and fermentation

Aspergillus ochraceus AUMC15539 with the registered accession number (OR346142), and *Fusarium proliferatum* AUMC15541 (OR346141) (Abdelrahem *et al.*, 2023; 2024) were used in the present investigation. A large-scale solid-state fermentation was utilized for the production of *Aspergillus*-*Fusarium* (AF) co-culture extract in Erlenmeyer flasks of 1L each containing autoclaved 100 g of rice with 110 mL of distilled water and autoclaved (Al Mousa *et al.*, 2021). After incubation for one month, flasks were extracted thrice using ethyl acetate (Mohamed *et al.*, 2021), filtered and then dried. The yield was determined and kept for further practices.

2.2 Impact of fungal co-culture extracts in controlling F. proliferatum pathogenicity on onion plants in the greenhouse

Greenhouse experiment was carried out and designed according to Riaz *et al.* (2010) protocol. Clean seedling plastic bags were prepared and filled with a mixture of sterilized soil: sand (1:3) at a rate of two kg/bag.

2.3 Artificial infection and treatment with fungal extracts and fungicide

Fusarium proliferatum inoculum was cultured on barley-washed sand (1:1 w/w) and incubated at 28±2 °C for 15 days, then the inoculum was mixed with sterilized soil at 2% (w/w). Seedling plastic bags were watered and left for one week to establish the fungal inoculum. Fungal co-culture extracts (1 g/L water) and the chemical fungicide (Bellis 38%) at a recommended dose (1 g/L water) were used as treatments. Onion transplants (Sabeeni variety) of uniform size, susceptible to basal rot disease, were surface sterilized with 1% sodium hypochlorite and thoroughly washed with sterilized water. The roots and stems of onion transplants were soaked in fungal extracts and chemical fungicide for 15 minutes before transplantation. A set of onion transplants, not treated with fungicides or fungal extracts, were prepared as an infectious witness (infected control), while another set, without fungal infection, was prepared as a healthy witness (healthy control). The pots were divided into groups according to each treatment (Hassanein *et al*., 2010). The pots were irrigated as it was necessary and fertilized as recommended.

2.4 Determination of fungal densities

Soil dilution and plate count technique was followed to determine the total fungal and *F. proliferatum* densities in the soil of the different treatments according to the method of Johansen *et al*. (1960). The plates were examined, the fungal colonies were counted, and the fungal densities were calculated.

2.5 Evaluation parameters

Measurements of several parameters were performed to estimate the response of the onion plants to different treatments. Damage reduction rate (R% of leaves and root dry weights) was calculated according to the following equation:

$$R\% = \frac{(DWA-DWP)}{DWA} \times 100 \quad (1)$$

Where DWA represents the dry weight of treated plants and DWP represent the dry weight of infected control.

The influence of the antagonist solely on the plant (D%) was examined as the development rate of the dry weight according to the following equation:

$$D\% = \frac{(DWA-DWP)}{DWP} \times 100 \quad (2)$$

Where DWA represents the dry weight of treated plants and DWP represents the dry weight of healthy plants as described by Boughalleb-M'hamdi *et al*. (2018).

Disease incidence and plant mortality were recorded after 10 weeks of transplantation (Ajmal *et al.,* 2001) according to the following equation 3:

$$DI\% = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100 \quad (3)$$

2.6 Morphological parameters

Plant height, root length, leaves length, fresh weights, and dry weights were determined and recorded (Metwally and Al-Amri, 2020).

2.7 Determination of photosynthetic pigments content

The pre-weighed samples of onion tubular leaf were impregnated in ethanol (20 mL/gram), homogenized using a homogenizer at 1000 rpm for about 5 minutes, and filtered using a cheesecloth. The obtained extracts were centrifuged at 5000 rpm for 10 min, the supernatants were separated, and absorbances were read at 400–700 nm using UV-VIS spectrophotometer for chlorophyll a at 666 nm, for chlorophyll b at 653 nm, and for carotenoids at 470 nm. The amounts of present pigments were calculated according to the formulas of Lichtenthaler and Wellburn (1983).

$$\text{Chlorophyll a} = 15.65\ A_{666} - 7.340\ A_{653} \quad (4)$$

$$\text{Chlorophyll b} = 27.05\ A_{653} - 11.21\ A_{666} \quad (5)$$

$$\text{Carotenoids} = 1000\ A_{470} - 2.860\ (\text{Chl. a}) - 85.9\ (\text{Chl. b})/245 \quad (6)$$

$$\text{Total pigments} = \text{chlorophyll a} + \text{chlorophyll b} + \text{carotenoids} \quad (7)$$

The pigment content was expressed as mg/g of fresh weight by the following equation:

$$\text{Chl. a} = \frac{\text{Chl.a } (\mu g/mL) \times \text{Extract volume (mL)}}{\text{Fresh weight of sample (g)} \times 1000} \quad (8)$$

2.8 Determination of biochemical compounds

2.8.1 Estimation of carbohydrates

Anthrone-sulphuric acid method depicted by Fales (1951) was adopted for carbohydrates determination, where 100 mg of fresh leaves were soacked in 5 mL of 2.5 N-HCl, hum for 3 hours in water bath, followed by sodium carbonate neutralization, and then filtered. One mL of filtrate was mixed with 5 mL of freshly prepared Anthrone reagent, kept at 90 °C for 10 minutes, and then tubes were cooled to room temperature. The absorbance was measured at 620 nm against a blank and the amount of carbohydrates was expressed as glucose equivalent (mg/g) using a standard curve of glucose.

2.8.2 Determination of protein content

The Lowry method (Lowry, 1951) was utilized to estimate total protein by centrifugation of leaf extract (100 mg leaf samples in 10 mL of sodium phosphate buffer (pH 7.5)) at 10,000 rpm for 10 min. 0.1 mL of the supernatant was diluted to 1 mL, mixed with 1 mL of reagent C (1 mL of copper sulfate (0.5%) and 50 mL of sodium carbonate (2 g), 0.4 g of sodium hydroxide (0.1 mol /L), and 1 g of sodium potassium tartrate dissolved in 100 mL of distilled water), and shake for 10 min. Consequently, 0.1 mL of 50% Folin–Ciocalteu reagent was added, and samples were left at room temperature for 30 min. After that, the absorbance was measured at 650 nm. The total protein was expressed as BSA equivalent (mg/g) using a standard curve of Bovine serum albumin.

2.8.3 Estimation of total phenolic and flavonoid contents

The total phenolic content of PCE extract was amounted according to Kupina *et al.* (2018). milled onion leaves (0.1 g) were homogenized in 10 mL of 70% acetone, then centrifuged at 5000 rpm for 10 min, and 1 mL of supernatant was mixed with 2.5 mL Folin–Ciocalteu reagent and one mL sodium carbonate. The mixture was vortexed, incubated at room temperature for 30 min in dark. After that, using UV-visible spectrophotometer, sample absorbance was measured at 750 nm. The phenolic content was expressed as gallic acid equivalent (GAE mg/g) using a standard curve equation. Meanwhile, measuring the total flavonoids content was performed by homogenization of milled onion leaves (0.1 g) in 10 mL of 80% ethanol, centrifuged at 5000 rpm for 10 min, and 0.5 mL of each extract was mixed ethanolic solution of $AlCl_3.6H_2O$. After 10 min, the sample absorbance was measured at 430 nm (Quettier-Deleu *et al.*, 2000). The flavonoid content was expressed as quercetin equivalent (QE mg/g) using a standard curve equation.

2.9 Statistical analysis

Data were displayed as mean±SE and demonstrated by analysis of variance (one-way ANOVA) using the SPSS software, version 16 (IBM, Armonk, NY, USA) with multiple comparison tests (Duncan) as being below the 0.05 level of significance. Principal component analysis (PCA) was carried out using the graphical presentation was obtained using Origin 2018 software (USA, Origin Lab).

3. Results

The study comprised the treatment of *F. proliferatum*-infected onion transplants, with *Aspergillus*-*Fusarium* co-culture and their mono-culture extracts along with commercial fungicide. After that, several significant parameters were determined including damage reduction rate, root and leaf length, fresh and dry weight, photosynthetic pigments, and biochemical compounds.

3.1 Determination of the microbial densities in the soil

The treatment with the AF co-culture extract presented significant reduction, superior to mono-cultures, in the total fungal (11.33×10^3) and *F. proliferatum* (3.67×10^3) counts and exhibited significant reduction as compared with the non-infected plants and Bellis (Figure 1).

3.2 Disease incidence and damage rate

The efficacy of AF co-culture and its monocultures on infected onion plants showed that extract of AF co-culture significantly decreased the disease index (DI) and demonstrated a mean infection frequency of 0.0% compared to 13.30% and 6.67% in Bellis treatment and healthy control, respectively. The damage reduction rate was maximum for AF extract (84%), while Bellis treatment reported a damage reduction rate of 59%. The extracts' impact was less noticeable for *Aspergillus* mono-culture extract with values of 4%. The AF co-culture exhibited the highest development rate (D%), reaching 68% of the total shoot and root dry weights, thus reflect the best behavior of AF extract treatment (Figures 2 and 3). Infected control plants showed 100% disease incidence, where the bulb was soft, irregular in shape, and discolored at the basal plate with a reduction of 50% in height and 60% in weight compared to the healthy control. Thus, AF extract offered sustainable superiority over other treatments.

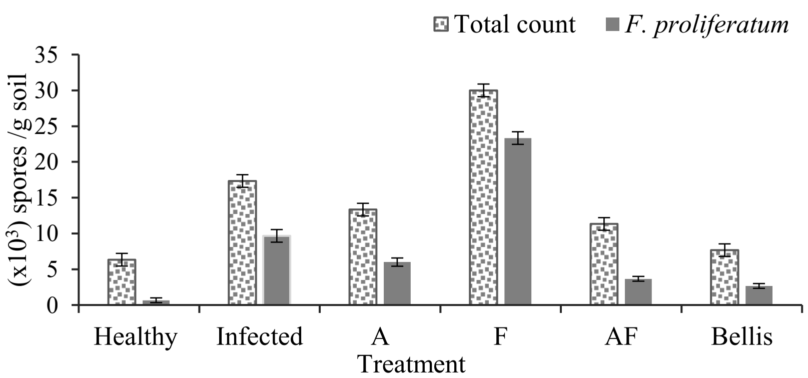

Figure (1): Influence of different treatments on total count of *F. proliferatum* and fungi in the onion plants rhizosphere.

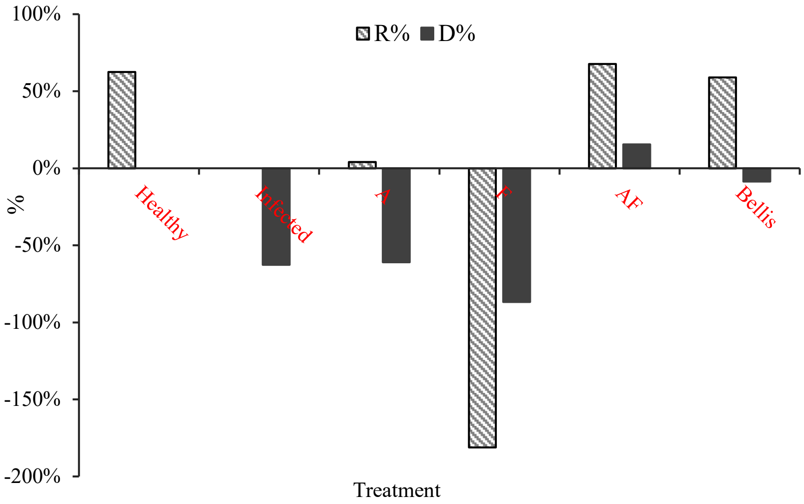

Figure (2): The damage redaction and development rate of the dry leaves and root weights.

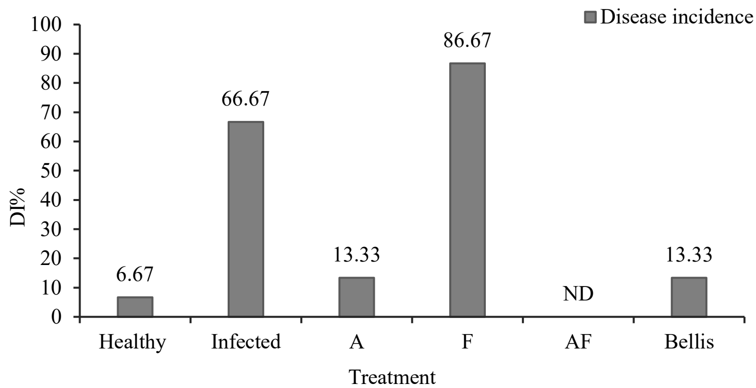

Figure (3): The influence of fungal extracts of single- and co-cultures, and Bellis on onion basal rot, disease incidence (%), under greenhouse conditions.

3.3 Growth parameters

Growth parameters included total plant height (TH), leaf height (LH), root height (RH), total plant fresh weight (TFW), leaf fresh weight (LFW), root fresh weight (RFW), total plant dry weight (TDW), leaf dry weight (LDW), and root dry weight (RDW) (Figure 4). The effectiveness of AF co-culture extract treatment revealed a significant increase in all parameters, offering reasonable superiority over other treatments, for the whole plant, root, and shoot with TH (59.67 cm), LH (36.00 cm), RH (23.67 cm), TFW (16.12 g), LFW (13.05 g), RFW (3.07 g), TDW (4.84

g), LDW (4.19 g), and RDW (0.35 g). Low effects on plant parameters were found in treatments with monocultures A and F extracts compared with healthy control.

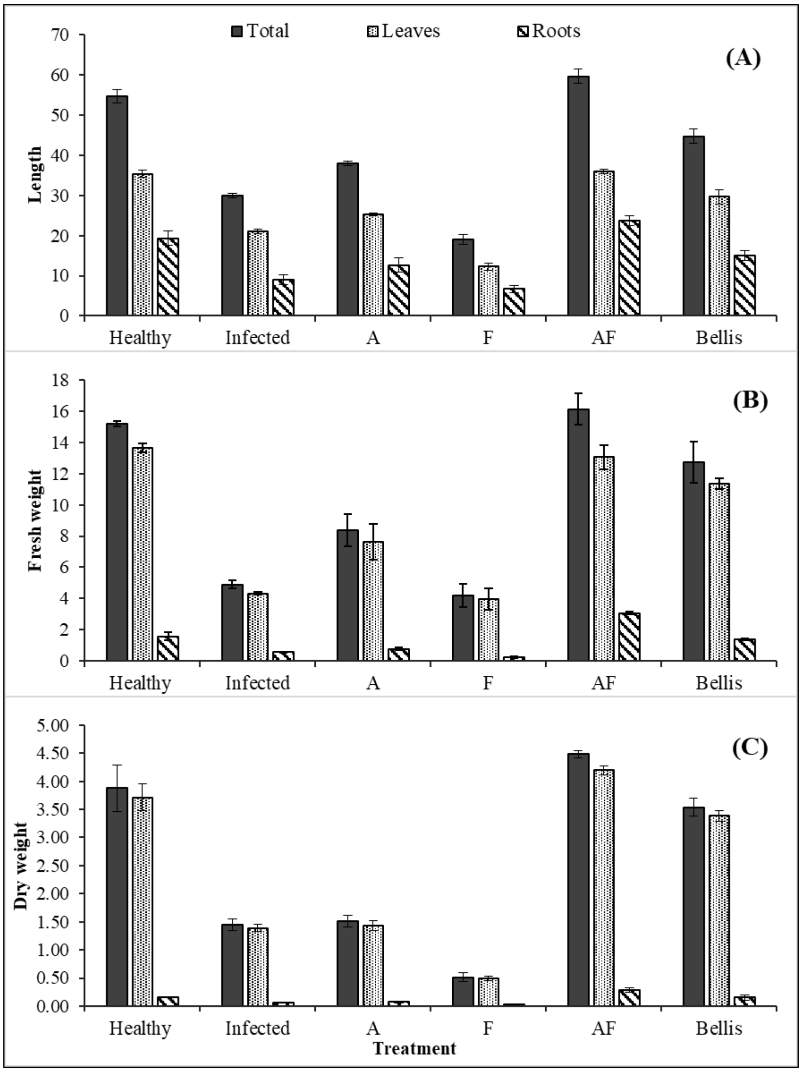

Figure (4): The influence of fungal extracts of single- and co-cultures, and Bellis on the growth parameters of infected onion plants (A; height, B; fresh weight, and C; dry weight).

3.4 Photosynthetic pigments content

The highest total pigment content (3.24 mg/g) was noticed with AF co-culture extract treatment, followed by the Bellis treatment (3.18 mg/g). The AF co-culture treatment scored the highest chlorophyll a content (1.63 mg/g), followed by the healthy control (1.59 mg/g), while the infected control exhibited the lowest chlorophyll a content (1.20 mg/g). The treatment with Bellis led to the higher chlorophyll b content (1.21 mg/g), followed by healthy control (1.07 mg/g), and the AF co-culture treatment (1.00 mg/g), while the infected control displayed the lowest chlorophyll b content (0.80 mg/g). Treatment with AF culture extract showed the highest carotenoid content (0.61 mg/g), followed by healthy control (0.58 mg/g) and Bellis displayed a moderate level (0.55 mg/g). The infected control displayed the lowest carotenoid content (0.45 mg/g) (Figure 5).

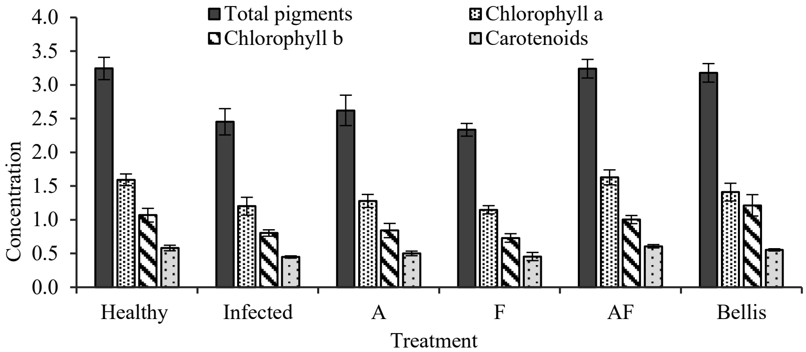

Figure (5): Single- and co-cultures extracts impact on total pigments, chlorophylls a and b, and carotenoids.

3.5 Biochemical compounds

It was worth notable that the AF co-culture extract demonstrated the highest efficacy in increasing the total carbohydrates of onion leaves (48.95 mg/g dry weight). Furthermore, the protein content of onion leaves was raised by the treatment with AF extract (105.23 mg/g), while the lowest protein content was detected in the infected control treatment (75.00 mg/g) (Figure 6). The phenolic content of leaves treated with AF co-culture extract was higher than with mono-culture extract treatments with a maximum increase of 41.64 mg/g, followed by the chemical fungicide and healthy control (31.45 and 31.93 mg/g, respectively), meanwhile the infected control reported the lowest phenolic content (27 mg/g). Total flavonoids maximum yield was detected with the AF extract (8.03 mg/g), followed by the healthy control (7.85 mg/g). The lowest flavonoid content was detected in the infected control (7.15 mg/g) (Figure 6). Figure (7) revealed a heat map of *F. proliferatum* infected onion transplants response to different treatments including mono- and co-culture extracts and commercial fungicide regarding soil microbial densities, disease incidence, morphological growth parameters, photosynthetic pigments, and biochemical compounds. Plots with red color refers to high response of plant estimated parameters to various investigated treatments. The AF co-culture extract recorded the highest positive impact on *Fusarium*-infected onion regarding damage reduction, development rate, plant height, fresh weight, dry weight, total pigments, carbohydrates, proteins, phenolic, and flavonoids, while reduction of total fungal count and *F. proliferatum* count reduced significantly with AF co-culture treatment.

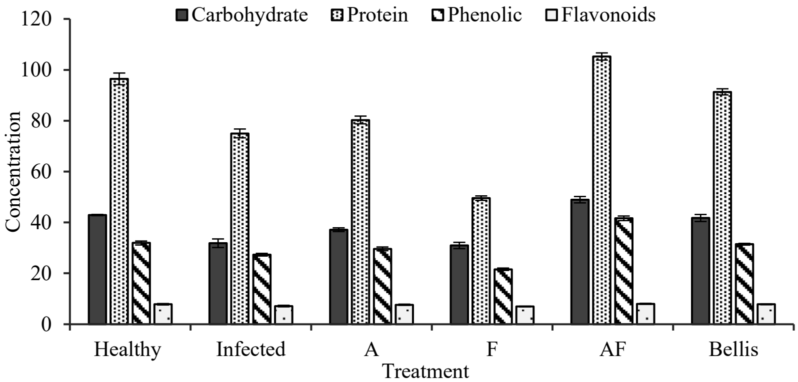

Figure (6): Effect of single- and co-cultures extracts, and Bellis on total biochemicals contents in infected onion plants.

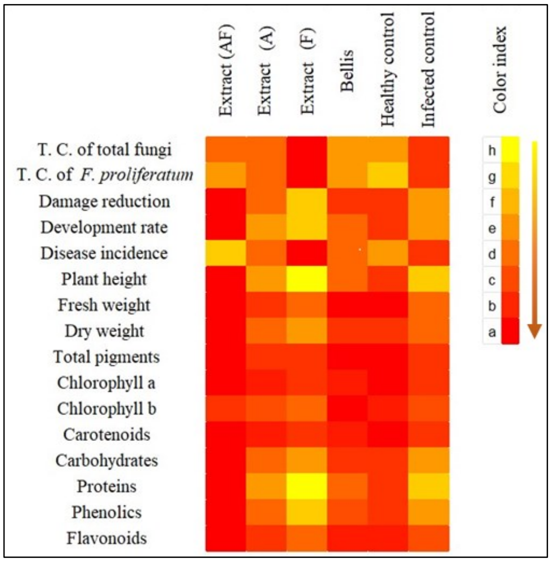

Figure (7): A heat map of *F. proliferatum* infected onion transplants response to different treatments in greenhouse experiments.

The principal component analysis revealed two components with eigenvalues greater than one, with group factors correlated with fungal and *F. proliferatum* counts, plant weight, pigments, carbohydrates, proteins, phenolics, and flavonoids. The first factor accounted for 75.3% and the second factor was 7.0%. Factor 1 showed a high loading linked to fungal densities, and dry weight. Carotenoids, carbohydrates, chl. a, phenolics, flavonoids, and leaf and root dry weight possessed strong positive loadings, while dry weight, chl. b, and total proteins had a negative correlation. Components with factor 2 high loadings comprised fungal counts. PC1, accounting for a significant portion of the variance in the indices of microbial densities, dry weight, and photosynthetic pigments, was influenced by monoculture and co-culture extracts (Figure 8). On the other hand, AF co-culture, Bellis treatment, and healthy control were substantially linked with growth parameters, photosynthetic pigments, and phytochemical constituents; these clusters were illustrated by the PC1 and PC2 high positive values.

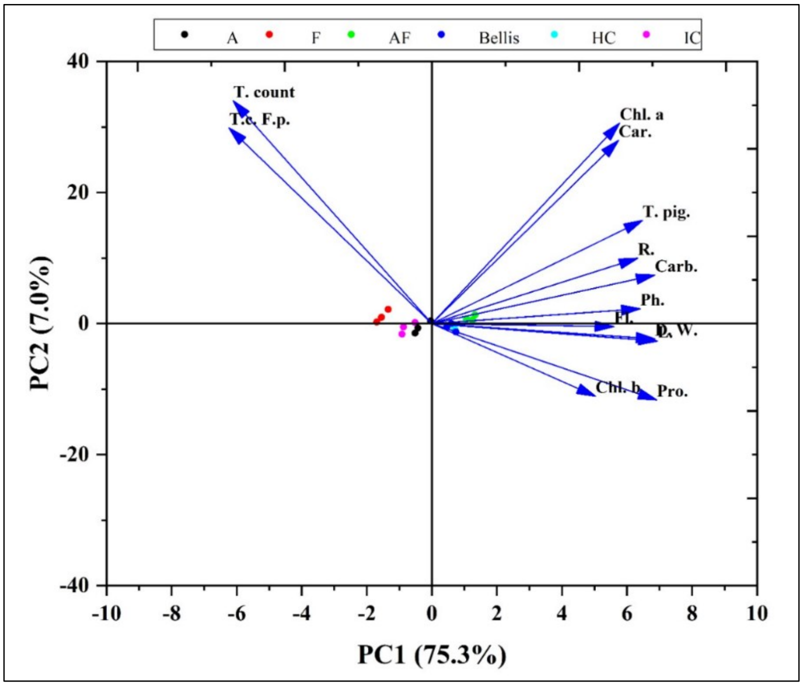

Figure (7): PCA score plot of the data set derived from diverse treatments on investigated parameters in greenhouse artificially infected onion plants.

4. Discussion

The co-culturing of two fungal species has been an efficient approach to induce the accumulation of new bioactive secondary metabolites (Marmann *et al.*, 2014). Co-cultivation with endophytic fungi can avoid biotic stress antagonistically through direct or indirect methods (Shinwari *et al.*, 2019), where direct antagonism relates to the biosynthesis of special metabolites enabling the decline of the phytopathogen population surrounding the host plant, while the indirect mechanism aids crop resistance improvement against phytopathogens (Pundir and Jain, 2015). Our study concerned to evaluate the potency of *Aspergillus*-*Fusarium* co-culture along with *Aspergillus* and *Fusarium* axenic cultures extracts to monitor *F. proliferatum*-causing onion basal rot experimentally in a greenhouse. The results established that the AF co-culture extract exerted significant reduction in onion FBR incidence as well as improving growth parameters of infected onion. The AF co-culture extract decreased the total fungal and *F. proliferatum* counts and reduced the percentage of infected plants to 0% compared to Bellis treatment (13.30%) and healthy control (6.67%), thus proves a strong suppression efficiency of AF co-culture extract against *F. proliferatum*. Biological control agents can be helpful in decreasing the soil inoculum potential of soilborne pathogens and therefore improve soil health and overall health of plants (Joshua and Mmbaga, 2020). Ghanbarzadeh *et al*. (2014) reported that fungi inhibit *Fusarium proliferatum* growth not only through rapid proliferation but also via bioactive compounds production. *Aspergillus ochraceus* has intermediate antifungal effects against various fungi, including *A. alternata, B. cinerea, F. oxysporum,* and *F. solani* (Morales-Sánchez *et al.*, 2021). Karim *et al*. (2022) proved that mixed fermentation of *Aspergillus tubingensis* and *Trichoderma asperellum* suppressed *F. oxysporum*, while Reis *et al*. (2020) indicated that *Aspergillus flavus* outcompetes *Fusarium verticillioides* in maize. Regarding onion growth parameters, the infected onion plants treated with AF co-culture revealed significant amelioration in all growth parameters with values higher than monocultures extracts and the healthy control. In addition, photosynthetic pigments were enhanced by AF co-culture extract treatment assuring the co-culture efficiency in boosting plant vitality and development. The AF co-culture treatment resulted in increasing the total carbohydrates of onion leaves (48.95 mg/g dry weight), protein content (105.23 mg/g), phenolic content (41.64 GAE mg/g), and flavonoids (8.03 QE mg/g) over other treatments. Endophytic aspergilli are promising reservoirs for bioactive compounds (Sharaf *et al.*, 2022). The utilization of endophytic *Aspergillus* on infected plants led to a noteworthy augmentation in the levels of photosynthetic pigments, total proteins, total carbohydrates, and total phenols when compared with the infected control plants that were not treated (Attia *et al.*, 2024). Plant and fungal extracts have been shown to be effective in many plants at inducing systemic resistance, reducing disease incidence, and improving plant growth and production (Hussein *et al.*, 2018). Principal component analysis revealed that the AF co-culture extract treatment was substantially correlated with growth parameters, photosynthetic pigments, and phytochemical constituents. The PCA is a statistical factorial analysis method permits the reduction of a set of unrelated variables into a smaller number of dimensions (Pessel and Balmat, 2008). The dimensions of the data space exceed the number of characteristic variables necessary to describe these data as there are correlations between the descriptive variables of data distribution. The higher the correlations between data descriptive variables, the smaller the number of useful characteristic variables for their representation (Konishi *et al.*, 2015).

5. Conclusion

The co-culture of *A. ochraceus* and *F. proliferatum* successfully enhanced the potent antifungal activities against *F. proliferatum*, making it a promising candidate for biocontrol applications. The significant improvements in antifungal efficacy, phytochemical content, and plant growth parameters demonstrated the potential of co-culture extracts as natural alternatives to conventional fungicides. Further research and development could lead to the commercialization of these extracts, providing sustainable and eco-friendly solutions for managing phytopathogenic fungi in agricultural settings.

References

Abdeen, M. A., Abd El mobdy, H. S., Hussein, S. M. and Hassane, A. M. A. (2024), "Single cell protein production from some food wastes using yeasts", *Archives of Agriculture Sciences Journal*, Vol. 7 No. 3, pp. 10–22.

Abdelrahem, M. M. M., Abouelela, M. E., Abo-Dahab, N. F. and Hassane, A. M. A. (2024), "*Aspergillus*-*Penicillium* co-culture: An investigation of bioagents for controlling *Fusarium proliferatum*-induced basal rot in onion", *AIMS Microbiology*, Vol. 10 No. 4, pp. 1024–1051.

Abdelrahem, M. M. M., Hassane, A. M. A., Abouelela, M. E. and Abo-Dahab, N. F. (2023), "Comparative bioactivity and metabolites produced by fungal co-culture system against myco-phytopathogens", *Journal of Environmental Studies*, Vol. 31 No. 1, pp. 1–15.

Abdou, H. A. A., Hassane, A. M. A., Abdel-Sater, M. A. and El-Shanawany, A. A. (2024), "Screening the oleaginous capacity of fungi from different habitats", *Journal of Environmental Studies*, Vol. 36 No. 1, pp. 101–115.

Abo-Dahab, N. F., Abdel-Hadi, A. M., Abdul-Raouf, U. M., El-Shanawany, A. A. and Hassane, A. M. A. (2016), "Qualitative detection of aflatoxins and aflatoxigenic fungi in wheat flour from different regions of Egypt", *IOSR Journal of Environmental Science, Toxicology, and Food*

Technology, Vol. 10 No. 7–II, pp. 20-26.

Ajmal, M., Ahmad, S. and Hussain, S. (2001), "Effect of soil moisture on black scurf disease and yield in potato", *Pakistan Journal of Biological Sciences*, Vol. 4 No. 2, pp. 175–201.

Aktar, W., Sengupta, D. and Chowdhury, A. (2009), "Impact of pesticides use in agriculture: their benefits and hazards", *Interdisciplinary Toxicology*, Vol. 2, pp. 1–12.

Al Mousa, A. A., Abo-Dahab, N. F., Hassane, A. M. A., Gomaa, A. F., Aljuriss, J. A. and Dahmash, N. D. (2022a), "Harnessing *Mucor* spp. for xylanase production: Statistical optimization in submerged fermentation using agro-industrial wastes", *BioMed Research International*, Vol. 2022, Article No. 3816010.

Al Mousa, A. A., Abouelela, M. E., Al Ghamidi, N. S., Abo-Dahab, Y., Mohamed, H., Abo-Dahab, N. F. and Hassane, A. M. A. (2024a), "Anti-staphylococcal, anti-*Candida*, and free-radical scavenging potential of soil fungal metabolites: A study supported by phenolic characterization and molecular docking analysis", *Current Issues in Molecular Biology*, Vol. 46, pp. 221–243.

Al Mousa, A. A., Abouelela, M. E., Hassane, A. M. A., Al-Khattaf, F. S., Hatamleh, A. A., Alabdulhadi, H. S., Dahmash, N. D. and Abo-Dahab, N. F. (2022c), "Cytotoxic potential of *Alternaria tenuissima* AUMC14342 mycoendophyte extract: A study combined with LC-MS/MS metabolic profiling and molecular docking simulation", *Current Issues in Molecular Biology*, Vol. 44, pp. 5067–5085.

Al Mousa, A. A., Abouelela, M. E., Mansour, A., Nasr, M., Ali, Y. H., Al Ghamidi, N. S., Abo-Dahab, Y., Mohamed, H., Abo-Dahab, N. F. and Hassane, A. M. A. (2024b), "Wound Healing, Metabolite Profiling, and In Silico Studies of *Aspergillus terreus*", *Current Issues in Molecular Biology,* Vol. 46, pp. 11681–11699.

Al Mousa, A. A., Hassane, A. M. A., Gomaa, A-E. R. F., Aljuriss, J. A., Dahmash, N. D. and Abo-Dahab, N. F. (2022b), "Response-surface statistical optimization of submerged fermentation for pectinase and cellulase production by *Mucor circinelloides* and *M. hiemalis*", *Fermentation*, Vol. 8, Article No. 205.

Al Mousa, A. A., Mohamed, H., Hassane, A. M. A. and Abo-Dahab, N. F. (2021), "Antimicrobial and cytotoxic potential of an endophytic fungus *Alternaria tenuissima* AUMC14342 isolated from *Artemisia judaica* L. growing in Saudi Arabia", *Journal of King Saud University-Science*, Vol. 33, Article No. 101462.

Attia, M. S., Salem, M. S. and Abdelaziz, A. M. (2024), "Endophytic fungi *Aspergillus* spp. reduce fusarial wilt disease severity, enhance growth, metabolism and stimulate the plant defense system in pepper plants", *Biomass Conversion and Biorefinery*, Vol. 14, pp. 16603–16613.

Boughalleb-M'hamdi, N., Salem, I. B. and M'hamdi, M. (2018), "Evaluation of the efficiency of *Trichoderma*, *Penicillium*, and *Aspergillus* species as biological control agents against four soil-borne fungi of melon and watermelon", *Egyptian Journal of Biological Pest Control*, Vol. 28 No. 1, pp. 1–12.

Cramer, C. S. (2000), "Breeding and genetics of *Fusarium* basal rot resistance in onion", *Euphytica*, Vol. 115, pp. 159–166.

Fales, F. (1951), "The assimilation and degradation of carbohydrates by yeast cells", *Journal of Biological Chemistry*, Vol. 193 No. 1, pp. 113–124.

Fan, C. M., Xiong, G. R., Qi, P., Ji, G. H. and He, Y. Q. (2008), "Potential biofumigation effects of *Brassica oleracea* var. *caulorapa* on growth of fungi", *Journal of Phytopathology*, Vol. 156 No. 6, pp. 321–325.

Gebretsadik, K. and Dechassa, N. (2018), "Response of onion (*Allium cepa* L.) to nitrogen fertilizer rates and spacing under rain fed condition at Tahtay Koraro, Ethiopia", *Scientific Reports*, Vol. 8, Article No. 9495.

Ghanbarzadeh, B., Goltapeh, M. E. and Safaie, N. (2014), "Identification of *Fusarium* species causing basal rot of onion in East *Azarbaijan province*, Iran and evaluation of their virulence on onion bulbs and seedlings", *Archives of Phytopathology and Plant Protection,* Vol. 47 No. 9, pp. 1050–1062.

Giavasis, I. (2014), "Bioactive fungal polysaccharides as potential functional ingredients in food and nutraceuticals", *Current Opinion in Biotechnology*, Vol. 26, pp. 162–173.

Hassane, A. M. A., Abo-Dahab, N. F., El-Shanawany, A. A., Abdel-Hadi, A. M., Abdul-Raouf, U. M., Ali, A. M. and Sultan, Y. Y. (2018), "*In vitro* and *in situ* impact of safe synthetic and natural antioxidants on populations and aflatoxin B_1 accumulation by *Aspergillus flavus*", *Journal of Biotechnology Science Research*, Vol. 5 No. 1, pp. 1–16.

Hassane, A. M. A., El-Shanawany, A. A., Abo-Dahab, N. F., Abdel-Hadi, A. M., Abdul-Raouf, U. M., Mwanza, M. (2017), "Cultural and analytical assays for aflatoxin B production by *Aspergillus flavus* isolates", *Journal of Natural Product Chemistry,* Vol. 1 No. 1, pp. 17–23.

Hassane, A. M. A., Hussien, S. M., Abouelela, M. E., Taha, T. M., Awad, M. F., Mohamed, H., Hassan, M. M., Hassan, M. H. A., Abo-Dahab, N. F. and El-Shanawany, A-R. A. (2022a), "*In vitro* and *in silico* antioxidant efficiency of bio-potent secondary metabolites from different taxa of black seed-producing plants and their derived mycoendophytes", *Frontiers in Bioengineering and Biotechnology*, Vol. 10, Article No. 930161.

Hassane, A. M. A., Taha, T. M., Awad, M. F., Mohamed, H. and Melebari, M. (2022b), "Radical scavenging potency, HPLC profiling and phylogenetic analysis of endophytic fungi isolated from selected medicinal plants of Saudi Arabia", *Electronic Journal of Biotechnology*, Vol. 58, pp. 37–45.

Hassane, A. M. A., Eldiehy, K. S. H., Saha, D., Mohamed, H., Mosa, M. A., Abouelela, M. E., Abo-Dahab, N. F. and El-Shanawany, A-R. A. (2024), "Oleaginous fungi: a promising source of biofuels and nutraceuticals with enhanced lipid production strategies", *Archives of Microbiology*, Vol. 206, Article No. 338.

Hassanein, N. M., Zeid, M. A. A., Youssef, K. A. and Mahmoud, D. A. (2010), "Control of tomato early blight and wilt using aqueous extract of neem leaves", *Phytopathology Mediterranean*, Vol. 49 No. 2, pp. 143–151.

Higginbotham, S. J., Arnold, A. E., Ibañez, A., Spadafora, C., Coley, P. D. and Kursar, T. A. (2013), "Bioactivity of fungal endophytes as a function of endophyte taxonomy and the taxonomy and distribution of their host plants", *PLoS ONE*, Vol. 8 No. 9, Article No. e73192.

Hussein, M. M. A., Abo-Elyousr, K. A. M., Hassan, M. A. H., Hashem, M., Hassan, E. A. and Alamri, S. A. M. (2018), "Induction of defense mechanisms involved in disease resistance of onion blight disease caused by *Botrytis allii*", *Egyptian Journal of Biological Pest Control*, Vol. 28, Article No. 80.

Johansen, L. E., Curi, E. A., Bonf, J. H. and Fribourg, H. A. (1960), *Methods for studying soil microflora plant disease relationship*, 2nd Eds., Burge's Publishing Co., Minneapolis, USA, p. 77.

Joshua, J. and Mmbaga, M. T. (2020), "Potential biological control agents for soilborne fungal pathogens in Tennessee snap bean farms", *HortScience*, Vol. 55 No. 7, pp. 988–994.

Karim, H., Azis, A. A. and Jumadi, O. (2022), "Antagonistic activity and characterization of indigenous soil isolates of bacteria and fungi against onion wilt incited by *Fusarium* sp.", *Archives of Microbiology*, Vol. 204 No. 1, pp. 1–9.

Khalaf, N. H., Hassane, A. M. A., El-Deeb, B. A. and Abo-Dahab, N. F. (2024), "Anti-candidal and antibacterial influence of zinc oxide nanoparticles biosynthesized by *Penicillium crustosum* AUMC15766", *Journal of Environmental Studies*, Vol. 36 No. 1, pp. 92–100.

Konishi, T. (2015), "Principal component analysis for designed experiments", *BMC Bioinformatics*, Vol. 16, pp. 1–9.

Kupina, S., Fields, C., Roman, M. C. and Brunelle, S. L. (2018), "Determination of total phenolic content using the Folin-C assay: single-laboratory validation, first action 2017.13", *Journal of AOAC International*, Vol. 101 No. 5, pp. 1466–1472.

Kwon, M. J., Steiniger, C., Cairns, T. C., Wisecaver, J. H., Lind, A. L., Pohl, C., Regner, C., Rokas, A. and Meyer, V. (2021), "Beyond the biosynthetic gene cluster paradigm: genome-wide coexpression networks connect clustered and unclustered transcription factors to secondary metabolic pathways", *Microbiology Spectrum*, Vol. 9 No. 2, Article No. e00898-21.

Lichtenthaler, H. K. and Wellburn, A. R. (1983), "Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents", *Biochemical Society Transactions*, Vol. 11 No. 5, pp. 591–592.

Lowry, O. H. (1951), "Protein measurement with the Folin-Phenol reagents", *Journal of Biological Chemistry*, Vol. 193, pp. 265–275.

Marmann, A., Aly, A. H., Lin, W., Wang, B. and Proksch, P. (2014), "Co-cultivation—a powerful emerging tool for enhancing the chemical diversity of microorganisms", *Marine Drugs*, Vol. 12, pp. 1043–1065.

Metwally, R. A. and Al-Amri, S. M. (2020), "Individual and interactive role of *Trichoderma viride* and arbuscular mycorrhizal fungi on growth and pigment content of onion plants", *Letters in Applied Microbiology*, Vol. 70 No. 2, pp. 79–86.

Mohamed, H., Awad, M. F., Shah, A. M., Nazir, Y., Naz, T., Hassane, A., Nosheen, S. and Song, Y. (2022a), "Evaluation of different standard amino acids to enhance the biomass, lipid, fatty acid, and γ-linolenic acid production in *Rhizomucor pusillus* and *Mucor circinelloides*", *Frontiers in Nutrition*, Vol. 9, Article No. 876817.

Mohamed, H., Hassane, A., Atta, O. and Song, Y. (2021), "Deep learning strategies for active secondary metabolites biosynthesis from fungi: Harnessing artificial manipulation and application", *Biocatalysis and Agricultural Biotechnology*, Vol. 38, Article No. 102195.

Morales-Sánchez, V., Díaz, C. E., Trujillo, E., Olmeda, S. A., Valcarcel, F., Muñoz, R., Andrés, M. F. and González-Coloma, A. (2021), "Bioactive metabolites from the endophytic fungus *Aspergillus* sp. SPH2", *Journal of Fungi*, Vol. 7 No. 2, pp. 1–12.

Omar, A. E., Al-Khalaifah, H. S., Mohamed, W. A. M., Gharib, H. S. A., Osman, A., Al-Gabri, N. A. and Amer, S. A. (2020), "Effects of phenolic-rich onion (*Allium cepa* L.) extract on the growth performance, behavior, intestinal histology, amino acid digestibility, antioxidant activity, and the immune status of broiler chickens", *Frontiers in Veterinary Science*, Vol. 7, Article No. 582612.

Oppong-Danquah, E., Budnicka, P., Blümel, M. and Tasdemir, D. (2020), "Design of fungal co-cultivation based on comparative metabolomics and bioactivity for discovery of marine fungal agrochemicals", *Marine Drugs*, Vol. 18 No. 2, Article No. 73.

Pessel, N. and Balmat, J. F. (2008), "Principal component analysis for greenhouse modelling", *WSEAS Transactions on Systems*, Vol. 7, pp. 24–30.

Pimente, M. R., Molina, G., Dionísio, A. P., Maróstica-Junior, M. R. and Pastore, G. M. (2011), "The use of endophytes to obtain bioactive compounds and their application in biotransformation process", *Biotechnology Research International*, Vol. 2011, Article No. 576286.

Pundir, R. K. and Jain, P. (2020), "Mechanism of prevention and control of medicinal plant-associated diseases", In: Kumar, V., Prasad, R., Kumar, M., *et al. Microbiome in Plant Health and Disease*, 1st Eds., Springer, Singapore, pp. 231–246.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J. C., Bailleul, F. and Trotin, F. (2000), "Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour", *Journal of Ethnopharmacology*, Vol. 72 No. 1–2, pp. 35–42.

Reis, T. A., Oliveira, T. D., de Zorzete, P., Faria, P. and Corrêa, B. (2020), "A non-toxigenic *Aspergillus flavus* strain prevents the spreading of *Fusarium verticillioides* and fumonisins in maize", *Toxicon*, Vol. 181 No. 1, pp. 6–8.

Riaz, T., Khan, S. N. and Javaid, A. (2010), "Management of *Fusarium* corm rot of gladiolus (*Gladiolus grandiflorus* sect. Blandus cv. Aarti) by using leaves of allelopathic plants", *African Journal of Biotechnology*, Vol. 9 No. 30, pp. 4681–4686.

Rongai, D., Pulcini, P., Pesce, B. and Milano, F. (2015), "Antifungal activity of some botanical extracts on *Fusarium oxysporum*", *Open Life Sciences*, Vol. 10, pp. 409–416.

Saber, S. M., Youssef, M. S., Arafa, R. F., and Hassane, A. M. (2016), "Mycotoxins production by *Aspergillus ostianus* Wehmer and using phytochemicals as control agent", *Journal of Scientific and Engineering Research*, Vol. 3 No. 2, pp. 198–213.

Selegato, D. M. and Castro-Gamboa, I. (2023), "Enhancing chemical and biological diversity by co-cultivation", *Frontiers in Microbiology*, Vol. 14 No. 2, pp. 1–24.

Sharaf, M. H., Abdelaziz, A. M., Kalaba, M. H., Radwan, A. A. and Hashem, A. H. (2022), "Antimicrobial, antioxidant, cytotoxic activities and phytochemical analysis of fungal endophytes isolated from *Ocimum basilicum*", *Applied Biochemistry* *and Biotechnology*, Vol. 194, pp. 1271–1289.

Shinwari, Z. K., Tanveer, F. and Iqrar, I. (2019), "Role of microbes in plant health, disease management, and abiotic stress management”, In: Kumar, V., Prasad, R., Kumar, M., et al. *Microbiome in Plant Health and Disease: Challenges and Opportunities,* 1st Eds., Springer, Singapore, pp. 231–250.