

Enhancing growth and suppressing root rot in *Phaseolus vulgaris* using ginger powder derived natural antifungal agent for optimum disease control and crop health

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Abstract

Chemical fungicides impart pollution, toxicity to non-target organisms and health risks. Utilization of plant derivatives hold potential as a valuable source of bioactive for both disease control and the promotion of plant growth. This study investigated the biological activity of *Zingiber officinale* (ginger) derived plant extract on *Ricinus communis* L. (castor), *Brassica* spp., and *Lantana camara* L., using different solvents for extraction against major root-rotting fungi, *Macrophomina phaseolina*, *Fusarium oxysporum* and *Aspergillus flavus*, using disc diffusion and agar well diffusion methods. At 100% concentration, both fresh and dried ginger extracts displayed total suppression (100%) of *M. phaseolina*, *F. oxysporum*, and *A. flavus*, with no apparent fungal growth. However, there was a little zone of inhibition (< 10%) for both dry, and fresh ginger, against *R. solani*. In comparison to the disc approach, the agar well method consistently yielded a great zone of inhibition. On the other hand, fungal overgrowth was noted, and no antifungal activity was demonstrated by ethanol-based extracts of *Lantana camara*, *Brassica nigra*, and *Ricinus communis*. When compared to untreated controls, *in vivo* tests showed that seeds treated with 100% fresh and dried ginger extracts dramatically increased root rot pathogen colonization by over 80% ($P < 0.01$). A substantial decrease in pathogen colonization was also seen after 100% extracts were soaked into the soil ($P < 0.05$). When ginger-treated seeds were combined with soil amendments, growth indices, including biomass, shoot length, and root length significantly enhanced. For instance, dry ginger + fudan resulted in 38% increase in shoot length, whereas dry ginger + DAP resulted in a 41% rise in root weight ($P < 0.05$). When soil amendment with 0.5 fresh ginger powder, the greatest decrease in fungal colonization (up to 95%) was observed. These results demonstrate the potential of ginger extracts, especially at 100% concentration, as efficient, environmentally friendly treatments for *Phaseolus vulgaris* L. root rot illnesses and enhancing plant development. Further research is recommended to optimize the application methods and explore the underlying mechanisms of ginger extract in promoting plant growth and disease suppression.

1.0 Introduction

Microbial invasion from virus to viroid, prokaryotic bacteria, eukaryotic fungi, oomycetes, and nematodes, causes significant crop damage annually during both pre-harvest and post-harvest phases (Jamiołkowska 2020; Debebe 2022). These plant diseases cause 40 billion dollars in consequential losses globally and are extremely persistent in their attack (Jamiołkowska 2020; Singh et al. 2023). Microbes related to plants can sustain several detrimental strategies by producing viroids, viruses, bacteria and fungi, and lead to infectious diseases affecting only the plant kingdom. Fungal disease frequency depends upon the plant health, the coincidence of a susceptible host, a virulent pathogen, and a favorable environment. The fungal disease invasion in plants also depends on the life strategy and the the pathogen population and the host plant change during their life cycle. The idea of biocontrol has sparked a significant political, economic, and technological discussion to create sustainable agriculture at a lower environmental cost (Barratt et al. 2018; Sun et al. 2025). Recent plant safeguarding tactics must immediately be based on natural resources due to the growing worries about ecological toxicity

and environmental pollution brought on by the careless use of chemical formulation (Jamiołkowska 2020; Jabeen et al. 2024).

Phaseolus vulgaris L. (red bean) is a herbaceous annual plant belongs to Leguminosae family known for their enriched macronutrients, micronutrients and antioxidant substances that nutrition for both humans and animals (Fernández-Ruiz et al. 2017; Chen et al. 2020). Various pathogenic fungi including, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium oxysporum*, and *Aspergillus flavus* cause huge economic losses to the bean plants. *R. solani* is the major root-rotting fungi, as found 91.8% during disease in bean plants (Mayo et al. 2015). This pathogen is more functional in disposed soil and at temperatures between 15 and 18°C. *R. solani* is a necrotrophic pathogen and one of the root and hypocotyl pathogens which help them in plant invasion that leads to alter plant physiology and destroy fruit yield as a consequence economic loss globally. *Macrophomina. phaseolina* is a soil-borne fungus that affects more than 500 plant species in around 100 families and causes charcoal rot, seedling blight, and root and stem rot (Ghosh et al. 2018). This fungus causes disease in soybeans, ground nut cultivars and sorghum under hot weather (30–35°C) and lower soil moisture (below 60%) (Marquez et al. 2021). *Fusarium* wilt, which is origin by *Fusarium oxysporum* and occurs in all regions, can also effects the productivity of common beans. *F.oxysporum* can be invaded through wounds, natural openings, or intact roots of bean plants with a preference for the tap root and lateral root interconnections (de-Borba et al. 2017). The most general fungus that causes contamination of food and feed and build aflatoxins is *Aspergillus flavus* (Lahouar et al. 2015). It is the source to cause contamination during food storage because it has the capacity to produce aflatoxins and its ability to survive as a pathogen and saprophyte in the food supply before and after harvest (Tai et al. 2020). *Aspergillus flavus* and *Aspergillus parasiticus* create secondary metabolites which are known for their high mutagenic, carcinogenic, and teratogenic properties (Shi et al. 2023).

Traditional Chinese utilized Ginger (*Zingiber officinale* Roscoe) as a source of spice and medication for more than 200 years. The ginger extract could be used as an additive in food and medical components due to their antifungal and therapeutic properties. Ginger essential oil and ginger extract both have antifungal activities as opposed to a variety of plant pathogens (Peng et al. 2022). Xi et al. 2022). The findings show that ginger contains phenolic compounds monoterpenoids, sesquiterpenoids, and their derivatives consist of ketones, aldehydes, alcohols, and esters. These chemicals offer a broad range of antimicrobial activity against various microorganisms, making them a promising substitute for synthetic microbial (Gao et al. 2022). Utilization of ginger could be an effective biostimulant as the extracted sap from them could be as disease protecting agents in many plant species and maintain the crop health of industrial importance. Natural ginger application could be used to enhance the productivity of crop plants and raised the life hood of developing nations and ensure food security and zero hunger.

Bioactive qualities found in *Ricinus communis* L., *Brassica nigra* (L.) Koch, and *Lantana camara* were selected in this study were based on optimum accumulation of bioactive qualities. Medicinal potential of *Ricinus communis* L. (castor) leaf, roots and seed oil was used as therapeutic uses have confirmed the larvicidal efficacy (Carolina et al. 2019). Likewise, essential oils (EOs) derived from mustard plants

(*Brassica nigra* L.), have potent antibacterial qualities that successfully prevent microbial growth and support both natural preservation and therapeutic applications (Mejía-Garibay et al. 2015). Shrub *Lantana camara* L belong to the genus *Lantana* contains over 150 species gain attention due to its medical potential and phytochemical richness. *Lantana camara* parts employed as biological control agents, stimulants, antibacterials, anti-inflammatory agents, and to treat rheumatism (Shi et al. 2023). These antifungal characteristics highlight the importance of these plants as tests to evaluate their biological activities using standardized extraction techniques for both *vitro* and *vivo* assessment. Using plants as fertilizers, those have higher antifungal activity are most reliable in contemporary agricultural production, have performed a fundamental role in enhancing crop yield and quality (Li et al. 2019; Akbar et al. 2024). Although unnecessary fertilization not only prevents the improvement of crop quality and yield, it also causes serious problems such as nutrient loss through leaching, hardness, and acidification of soil, crop pest annoyance, and groundwater safety issues (Du et al. 2021). The objective of the current study was to evaluate the assess the antifungal activity of ginger powder promotes growth and inhibits root-rotting fungi in *Phaseolus vulgaris* L. Study of such kind could be helpful to improve plant disease management techniques and offer an alternative to chemical fertilizers which not only increase production but also pose no environmental risks.

2.0 Materials and Methods

2.1 Collection of material and seeds

Fresh ginger rhizome and dry powder were collected from Mosamiyat, near University Road Karachi, Pakistan. The leaves of *Lantana camara* L. and castor (*Ricinus communis* L.) were gathered from the University of Karachi and leaves of mustard (*Brassica nigra* (L.) Koch) were collected from a local market near Mosamiyat, Karachi, Pakistan. Organic fertilizers, cow dung and goat dung, inorganic fertilizers fudan, and diammonium phosphate (DAP) were collected from a nursery near the University of Karachi. Red bean seeds were collected from New Sabzi Mandi Karachi, Pakistan, for pots experiment.

2.2 Preparation of stock solution of ginger, mustard, castor, and lantana camara L.

For the preparation of stock solution 10g of fresh and dry ginger powder were soaked in 100 mL of appropriate organic solvent (n-hexane). The flasks were covered with aluminum foil and left for 72 hours. Fresh leaves of mustard, castor, and *Lantana camara* dried under sunlight for 24 hours. For the preparation of stock solution, 10g of the tested leaves powder was weighed and added to the flask containing 100 mL of the appropriate organic solvent (ethanol), separately. Flasks were covered with aluminum foil and left for 1 week. The solutions were centrifuged at 3000 rpm for 10 minutes and used for *in-vitro* studies while residue was discarded (Michiels et al. 2012). The crude plant extract was considered as 100% stock concentration. One gram of crude extract diluted in one mL of solvent, for instance, equal 100% (w/v) stock.

2.3 The Isolation of Pathogenic Fungi

2.3.1 Isolation of *M. phaseolina* by using the wet sieving technique

Soil samples were taken from the rhizosphere of infected soybean plants exhibiting classic charcoal rot signs in the field station of the University of Karachi, Pakistan. In order to extract microsclerotia from the soil, the pathogen was separated by using the wet sieving approach, as outlined by Ghaffar (1976). In short, distilled water was used to suspend 100g of air-dried soil, which was then run through a succession of sieves with progressively smaller mesh sizes (60, 100 and 200). To stop bacteria from growing, the material that was left on the last sieve was cultivated on potato dextrose agar (PDA) treated with streptomycin and penicillin. Emerging fungal colonies were preliminarily identified as *Macrophomina phaseolina* based on morphological traits, including the formation of numerous black microsclerotia, absence of conidia, and fast-growing grey to black mycelia (Schafer & Kiewnick. 2010).

2.3.2 Isolation of *R. solani* by baiting technique

Samples of soil were taken from the rhizosphere of infected chilli (*Capsicum annuum*) plants from agricultural fields of the University of Karachi, Pakistan, that were exhibiting indications of basal stem rot. The soil plating method outlined by Anderson and Papavizas (1965) was used to recover the fungal isolate. Plates were incubated at $25 \pm 1^\circ\text{C}$ for 3–5 days after 10 g of soil was spread out on water agar (WA) supplemented with streptomycin sulphate (100 mg/L). For purification, hyphal tips from newly formed colonies were subcultured onto potato dextrose agar (PDA).

Preliminary identification of the fungal isolate as *Rhizoctonia solani* was based on morphological traits, including right-angled hyphal branching, absence of conidia, and presence of typical multinucleate cells seen under a compound microscope following safranin staining. DAPI staining was used to confirm whether the condition was binucleate or multinucleate. The internal transcribed spacer (ITS) region of rDNA was amplified using the universal primers ITS1 and ITS4 in order to perform molecular identification and verify the identity (White et al. 1990).

2.3.3 Soil dilution for the *F. oxysporum* isolation

Soil samples were taken from the rhizosphere of wilted tomato (*Solanum lycopersicum*) plants from agricultural fields of the University of Karachi, Pakistan, that displayed the typical indications of vascular wilt. According to Leslie and Summerell (2006), the pathogen was identified using the soil dilution plate approach. The fusarium- selective medium from Komada, which is known to promote the development of *Fusarium* spp. while inhibiting the growth of bacterial and other fungal pollutants, was plated onto 100 grams of air dried soil that had been serially diluted in sterile distilled water. Subculturing on potatoes dextrose agar (PDA) allowed for the purification of newly formed fungal colonies. Based on morphological features such as development of many microconidia, three to five septate sickle-shaped macroconidia, and thick walled chlamydospores seen under a compound microscope, *Fusarium oxysporum* was initially identified (Booth, 1971). On PDA, the colony morphology displayed cottony aerial mycelium and characteristic white to pinkish-purple coloring.

2.3.4 Isolation of *Aspergillus flavus*

In agricultural fields at the University of Karachi, Pakistan, an area known for frequent contamination by *Aspergillus*, soil samples were taken from the rhizosphere of ground nuts (*Arachis hypogaea*) plants. The dilution plate technique was used for isolation, in accordance with Pitt and Hocking's (2009) methodology. To promote specific growth, serial dilutions of air-dried soil were made in sterile distilled water and plated on semi-selective media such as *Aspergillus flavus* and *parasiticus* agar (AFPA) and Czapek- Dox agar (CDA). For five to seven days, plates were incubated at 28 ± 1 °C. To examine morphological traits, colonies that resembled *Aspergillus flavus* were subcultured on PDA. Both macroscopic and microscopic characteristics, such as yellow green conidial head, biseriate conidiophores, and the presence of rough walled conidia organized in chains, were used for the first time identification (Klich, 2002).

2.4 *In-vitro* experiment

2.4.1 Antifungal Activity using Paper Disc Diffusion Method

Sterilized Whatman's paper discs were soaked in stock solution of 100 µL of fresh and dried ginger (extracts separately), n-hexane (control for ginger), ethanol (control for castor, mustard, *Lantana camara*) and fungicide (carbendazim) for half an hour. Pour the petri plates with Potato Dextrose Agar (PDA) medium containing streptomycin 0.2/L and penicillin 100 units/L. Sterilized Whatman's paper discs soaked in respective solutions (castor, mustard, *Lantana camara*, fresh and dry ginger powder) were placed on three sides of petri plates. In the center of PDA poured petri plates, a disc of each fungus (*M. phaseolina*, *F. oxysporum*, *R. solani*, and *A. flavus*) was placed, and each fungus was replicated thrice. At 25–33°C temperature petri plates were incubated for 7 days. After an incubation period, the zone of inhibition was measured by scale in mm units (Silva & Domingues, 2017).

2.4.2 Antifungal activity using Agar well method

PDA medium having penicillin 100 units/L and streptomycin 0.2/L was poured into petri plates. By using a sterilized 5mm cork borer three wells were made in PDA poured petri plates, and each well was inoculated with each stock solution (castor, mustard, *lantana camara*, fresh and dry ginger powder), n-hexane (control for ginger), ethanol (control for castor, mustard, *Lantana camara*) and fungicide (carbendazim). Discs of fungus (i.e. *M. phaseolina*, *F. oxysporum*, *R. solani*, and *A. flavus*) was placed in the middle of PDA-poured petri plates. Each examined fungus was replicated three times. At 25–33°C Petri plates were incubated for 7 days. After an incubation period, the zone of inhibition was measured by scale in mm units (Espinell-Ingroff, et al. 2007).

The zone of growth inhibition was measured and calculated by the following formula.

$$X = \frac{Y}{Z} \times 100$$

X = percentage of growth inhibition

Y = Growth of mycelia measured in control plates (mm)

Z = Growth of mycelia measured in treated plates (mm)

2.5 *In-vivo* experiment

Fresh and dry ginger powder which showed effective results in the *in-vitro* studies among all other treatments (castor, mustard, and *lantana camara*) was carried to the in-vivo studies on red beans.

2.5.1 Collection of soil and seed treatment

Sandy loam soil obtained from a nursery near the University of Karachi, sieved the soil, and the debris was discarded. The soil was transferred into clay pots (600g/) pots. Red bean seeds were sterilized with 1% NaClO, using distilled water, cleaned twice, then allowed to air dry. Tested seeds were treated with 100 and 75% concentrations of both dry and fresh ginger powder separately for about half an hour while untreated seeds were used as control. Five seeds were planted in pots, and there were three replicates for each treatment. Infection percentage and growth parameters were documented after one month of seed germination.

2.5.3 Soil amendment and drenching

Each pot had 600 g of soil and was amended with dry and fresh ginger (0.1% and 0.5%) powder. After amendment for one day, five seeds of red beans after surface sterilization with 1% NaClO were planted in each pot. Three replicates were made for each treatment. After one month of seed germination, infection percentage and growth parameters were documented. For soil drenching, 75% extract is made by dilution of 25% of the stock, and 100% extract is undiluted stock. 25 mL prepared concentration of 75% and 100% of both fresh and dry ginger were drenched into clay pots having soil(600g) while untreated soil was used as control. Five seeds were planted in pots and, three replicates for each treatment. Growth parameters and percentage of infection were documented after one month of seed germination.

2.5.5 Growth experiment

Pots were filled with 600g of soil. Organic fertilizers (cow dung and goat dung) and inorganic fertilizers (DAP and fudan) amended in soil at 0.1 and 0.5% per pot. Tested bean seeds were treated with extracts of both fresh and dry ginger powder. Another experiment was set up of these fertilizers without treating seeds with ginger. After one day of amendment, 5 seeds were sown in each container having 600g soil, and were watered regularly. Growth parameters such as infection percentage, number of leaves and nodules, and shoot and root length and weight were recorded after 1 month of seed germination.

2.5.6 Colonization % of pathogenic fungi from roots

After one month of red bean growth, the plants were pulled out, the roots were cleaned with running water and dried with paper. The taproot of each treatment was cut into 5 small pieces and soaked in 1% of sodium hypochlorite NaClO for three minutes for surface sterilization, and washed with sterilized distilled water three times. After the roots were dried the fragments were put on a PDA-poured petri dish

having antibiotics (penicillin and streptomycin) to inhibit the growth of bacteria and incubated at room temperature for one week. Fungi that emerged from the root segment after incubation were examined under the microscope and the percentage of colonization was calculated by the following formula; $a = \frac{b}{c} \times 100$

a = Colonization percentage

b = Number of fungi colonized by root pieces

c = Total roots pieces

2.6 Statistical analysis

Data was reported as mean $\pm$ standard deviation. The zone of inhibition of fungi was expressed in percentage and represented by using a chord diagram. To examine the effects of seed treatment, soil drenching, and soil amendment and the treatments using IBM SPSS statistics 26.0, the principal component analysis was performed. With significance of 0.000, Barlett's Test of Sphericity confirms that Principal Component Analysis could be carried out on the data subjected to the analysis. The colonization percentage of the root-rotting pathogens was represented using a Heat map with labels. The graphs were plotted using OriginPro 2024b.

3.0 Experimental Results

3.1 *In-vitro* test

Two different methods (disc diffusion and agar-well method) were used to find the effect of extracts against the tested fungi namely, *M. phaseolina*, *R. solani*, *F. oxysporum*, and *A. flavus* (Fig. 1-Fig. 5S). Test fungi were suppressed and distinct zones of inhibitions were produced when extract at 100% concentration of both fresh ginger and dry ginger powder was used except for *R. solani*, which didn't show the zone of inhibition against fresh ginger extract. Dry ginger powder extract showed a significant zone of inhibition against *F. oxysporum* in the agar well method. However, fresh ginger extract showed a minimum zone of inhibition against *M. phaseolina*. The extracts of *Lantana camara*, mustard, and castor didn't show a zone of inhibition against the test fungi, however overlapping of test fungi over the extracts was observed.

Overall results showed the complete inhibition of *M. phaseolina*, *F. oxysporum*, and *A. flavus* by both ginger extracts but inhibition of the *R. solani* by both ginger extracts was negligible. Extracts of castor, *Lantana camara*, and mustard were not effective against tested fungi. Among both of the methods, the Agar well method showed the highest zone of inhibition followed by the paper disc method (Figs. 1 and 2).

3.2 *In-vivo* results

Principal component analysis

The principal component analysis was carried out on the physical parameters of red bean plants, including root length, root weight, shoot length, shoot weight, no. of leaves, no. of nodules, and dry root and shoot weight in the greenhouse experiment. Three different pot experiments were plotted. The first experiment shows the effect of fertilizer and fertilizer + seed treatment by fresh ginger on the growth of red beans and against root-rotting fungi. The second experiment shows the effect of fertilizer and fertilizer + seed treatment by dry ginger powder on the growth of red beans and against root-rotting fungi. The third pot experiment shows the effect of soil amendment, soil drenching, and seed treatment by fresh ginger and dry ginger powder on the growth of red beans and against root-rotting fungi. It demonstrates how organic amendment and other attributes influence heterogeneity compared to the controlled plants.

For the 1st pot experiment, a total of 76.98% combined variance was seen in the data set. Out of which, the 1st principal component gives 51.20% variance while 25.78% was shown by the 2nd principal component. The comparative distance of the treatments from the control on the score plot shows their positive correlation with one another (Supp. Figure 1). For the 2nd pot experiment, a total of 67.53% combined variance was seen in the data set. Out of which, the 1st principal component gives 44.30% variance while 23.23% was shown by the 2nd principal component. The comparative distance of the treatments from the control on the score plot shows their positive correlation with one another (Supp. Figure 2). For the 3rd pot experiment, a total of 55.18% combined variance was seen in the data set. Out of which, the 1st principal component gives 34.11% variance while 21.28% was shown by the 2nd principal component. The comparative distance of the treatments from the control on the score plot shows their positive correlation with one another (Supp. Figure 3). Different methods were used with fresh ginger and dry ginger powder and extract for the management of root pathogenic fungi, and to enhance the growth of red bean plants.

Red Bean seeds were treated with 75% ($P < 0.05$) of fresh ginger increased the number of leaves, shoot length, and shoot weight as compared to control. Similar results were also recorded for dry ginger powder. Seeds treated with 100% concentration of dry ginger powder was found to be best to improve the length and weight of bean plants. Soil 600g drenched with 100% concentration of dry ginger powder significant ($P < 0.01$) to improve the length and weight of red bean root. Pots drenched with 75% of dry ginger increased ($P < 0.05$) the shoot length and weight as compared to control. Similar results were also recorded for fresh ginger powder. Soil amended with 0.5% concentration of dry ginger powder was significantly ($P < 0.01$) enhanced the length and weight of roots of beans. Pots soil amended with 0.5% dry ginger showed an increase the number of leaves, length and weight of shoot followed by 0.1%. Similar results were also recorded in fresh ginger powder (Fig. 3). Among the three methods, all were found effective but seed treated at 100% concentration could be used as an inexpensive tool in commercial scale as compare to soil drenching and soil amendment method which is recommended for low scale farming.

By using organic fertilizers (goat dung and cow dung) and inorganic fertilizer (DAP) amended @0.1% along with seeds treated at 100% gave maximum increase in growth parameter of shoot and root of

beans plant and impressively reduced the infection of root rotting fungi. Shoot length was increased significantly ($P < 0.05$) when soil was amended with Fudan in combination with seed treatment with dry ginger powder. Shoot weight and root length increased significantly ($P < 0.05$) when soil was amended with cow dung in addition with seeds treated with dry ginger powder. Similarly, root weight was increased significantly ($P < 0.05$) when soil was amended with DAP in combination with seed treatment with dry ginger powder. Shoot weight and root length were increased significantly when soil was amended with goat dung in combination with seed treated with fresh ginger. Similarly, root weight increased significantly when soil was amended with cow dung and seed treated with fresh ginger. Shoot length was increased when soil was amended with fudan in combination with seed treatment with fresh ginger (Fig. 3–5).

Colonization percentage of root-rotting fungi (*M. phaseolina*, *R. solani*, *F. solani*, and *F. oxysporum*)

Combine effect of fertilizers with seed treatment by fresh and dry ginger.

Soil amended with fudan in combination with seeds treated with fresh ginger reduced ($P < 0.01$) the colonization percentage of root-rotting fungi. Similarly, soil amended with cow dung, DAP, fudan, and goat dung in combination with seeds treated with fresh ginger and dry ginger extracts were more effective against root-rotting fungi.

Seed treatment, Soil drenching, and Soil amendment method by fresh ginger and dry ginger

Sterilized seeds with 1% NaClO were treated by fresh ginger and dry ginger powder with 75% and 100% concentrations separately for 30 minutes. The seeds of red beans were sown in each pot. Each treatment was replicated three times. The seed not treated acts as control. The colonization percentage of root rot pathogen was reduced ($P < 0.01$) when seeds were treated with 100% of the extract of both fresh ginger and dry ginger powders. Seeds treated with 100% show maximum inhibition as compared to the control. Both treatments show antifungal activity against root-rotting fungi on bean plants. Sterilized seed after 1% NaClO were sowed in the soil, and the soil was drenched at 75% and 100% concentration of both tested ginger extracts. Each treatment was replicated thrice. Untreated seeds were served as control. The colonization percentage of root rot pathogens was reduced ($P < 0.05$) when soil was drenched with 100% extracts of both fresh ginger and dry ginger powder. Both treatments at different concentrations showed antifungal activity against root rotting fungi and improved the growth of red bean plants. Sterilized seeds with 1% NaClO were sowed in soil at different concentrations (0.5% and 0.1%); each treatment was replicated three times. The seed not treated act as control. The colonization percentage of root rot pathogen significantly decreased when soil was amended with 0.5 and 0.1% of fresh ginger powder. However, maximum inhibition ($P < 0.01$) of *M. phaseolina*, *F. solani*, *R. solani*, and *F. oxysporum* were recorded as 0.5% as compared to control. At different concentrations ($P < 0.01$) both fresh ginger and dry ginger powders showed antifungal activity against root rotting fungi by using soil amendment method experiment.

4.0 Discussion

Overuse of chemical fertilizers is a multilayered problem with severe consequences, adversely affecting human health and crop health. Extensive use of agrochemicals, in agriculture is inducing the chemical resistance in the phytopathogens against the commonly used chemical fertilizers in the field (Bódalo et al. 2023). The current research was designed to use organic material instead of chemicals to control root pathogenic fungi associated with beans. For this purpose, the antifungal effect of ginger, mustard, castor, and *Lantana camara* was accessed in the *in-vitro* experiment, and the effective organic material, *i.e.*, ginger, was carried to the *in-vivo* experimentation, where its effects on the growth parameters and root rot colonization were observed. One-third of the world's crop production is lost due to plant pathogens each year. It has been reported that the fungal pathogens cause severe damage among the biotic stresses, followed by bacterial and viral infections (Wani et al. 2023). Root rot, stem-blight, and stem-rot are the major diseases of common beans and cause huge economic losses.

According to Ali et al. (2022), ginger is rich source of bioactive substances with potent antifungal and antibacterial properties, including zingerone, shogaol, and gingerol. Present results showed that ginger extract in the *in-vitro* experiment showed the maximum zone of inhibition against test fungi due to the anti-microbial effect. Our results are also in concordance with Yadav et al. (2023) that ginger can stop the growth of some of the most prevalent fungal pathogens. Previous research also showed that ginger oil has been estimated to possess antimicrobial effects (Beristain-Bauza et al. 2019). The research carried out by Gao et al. (2022) reported that monoterpenoids, sesquiterpenoids, phenolic compounds and their derivatives, aldehydes, ketones, alcohols, and esters are present in ginger, which gives a broad antimicrobial array against various pathogens and makes it a potential alternative to synthetic fertilizers. The study carried out by Xi et al. (2022) reported the antifungal potential of ginger extract and ginger essential oil against *F. oxysporum*.

In the present research, castor, mustard, and *Lantana camara* didn't show effective results against the pathogenic fungi. However, various researchers have reported their antifungal activities. Carolina et al. (2019) have reported that ricinine is the main compound found in the castor leaves responsible for the toxic effects against various pathogens. Compound in *Ricinus communis* L.'s seeds, leaves, and oil are mostly responsible for its remarkable antifungal qualities. Ricinoleic acid, a fatty acid with well-known antibacterial properties, is present in castor oil. Numerous studies demonstrate its effectiveness against harm full fungi that affect both plants and humans, such as *Rhizoctonia solani*, *Aspergillus* species, and *Fusarium* species. Furthermore, Mejia-Garibay et al. (2015) reported that the essential compounds of mustard *i.e.*, allyl-isothiocyanate (AITC) shows antimicrobial activity, in both vapor and liquid phase against *Aspergillus niger*, *A. ochraceus* and *Penicillium citrinum*. Glucosinolates, which are abundant in *Brassica nigra*, break down into isothiocyanates, which are strong antifungal substances. Fungal cell membranes and metabolic processes are disrupted by these bioactives. Extract from mustard has demonstrated efficacy against fungi, including species of *Alternaria*, *Fusarium*, and *Botrytis*. Triterpenoids, flavonoids, and essential oils found in *Lantana camara* L. have broad-spectrum antibacterial properties. *M. phaseolina*, *F. oxysporum*, and *A. flavus* are among the phytopathogenic fungi whose growth has been demonstrated to be inhibited by its extracts. The disruption of fungal cell wall synthesis and mitochondrial dysfunction are probably the cause of the antifungal action (Shi, Y et al.

2023). Due to the efficient results of ginger in the *in-vitro* experiment, it was carried to the pot experiment where it successfully improved the physical parameters and suppressed the root-rotting systemic fungi. The research carried out by Ali et al. (2022) reported that the application of ginger extract enhanced damask rose growth characteristics and it is as a result in the availability of nutrients for enhanced growth. Similarly, Xi et al. (2022) reported that the constituents of ginger, including 4-hydroxybenzaldehyde and quercetin, inhibit the growth of *F. solani* completely and suggested their use as a natural fungicide in disease management. Additionally, investigations on plant-microbe interactions have shown that ginger powder may affect the dynamics of microorganisms in the soil, favoring beneficial bacteria while decreasing pathogens (Carolina et al. 2019). Increased nutrient absorption, better root system, and eventually higher crop output are the outcomes of the combined impact.

Generally, the colonization percentage of *M. phaseolina*, *F. solani* and *F. oxysporum* were more suppressed than *R. solani* when combined with fertilizers and seeds treated by fresh and dry ginger powder also improved the growth of red plants as compared to individual treatment. Overall result showed that root pathogenic fungi were more suppressed by ginger dry powder along with treatment with fudan and increased the plant growth as compared to other. Among three methods (seed treatment, soil drenching, and soil amendment), all were found effective but seed treated with 100% concentration could be used as an inexpensive tool on commercial scale as compare to soil drenching and soil amendment methods which is recommended for low scale farming. It is suggested from the present research that the application of fresh and dry ginger extracts should be used against root-rotting fungi and for the improvement of the growth of red bean plants on large agricultural fields, which is cheap, easily available and non-toxic to the soil environment. So, it is a useful alternative to fungicides and bactericides.

5.0 Conclusion

Based on the study on the application of ginger to enhance the growth of red beans and suppress root-rotting pathogenic fungi, the results suggest that ginger can be an effective organic amendment for improving the growth and health of red beans. The study found that the application of ginger extract improved the seed germination rate, plant growth, and yield of Red beans, while also suppressing the growth of root-rotting pathogenic fungi. This was attributed to the presence of various bioactive compounds in ginger, including gingerol and shogaol, which have been shown to have antifungal and plant growth-promoting properties. The use of organic amendments, such as ginger, can have several benefits for plant growth and soil health. Organic amendments can improve soil fertility, increase microbial activity, and suppress the growth of soil-borne pathogens. This can lead to improved plant growth, yield, and quality, while also reducing the need for synthetic fertilizers and pesticides. Overall, the use of organic amendments like ginger can be an effective strategy for improving the growth and health of Red beans, while also promoting sustainable agriculture practices. An efficient method for promoting *Phaseolus vulgaris* development and inhibiting root rotting fungal infections, including *M. phaseolina*, *F. oxysporum*, and *R. solani* is the application of powdered ginger. This purpose demonstrates that ginger (*Zingiber officinale*) promises a sustainable, natural, and environmentally friendly substitute for chemical

fungicides in the treatment of legume diseases. In order to maximize ginger powder's effectiveness, future studies should concentrate on adjusting its concentration and administration method under various agronomic circumstances. To evaluate the effect of recurrent ginger treatment on soil microbiota in group health, long-term research is also required. Additionally, combining ginger powder with other biological control medications may enhance its ability to inhibit illness.

Declarations

Ethics approval Not applicable

Consent to Participate We confirm that the manuscript has been read and approved by all named authors and that there are no other people who satisfied the criteria for authorship but are not listed. We further confirm that all the authors listed in the manuscript have been approved by all of us.

Authors contribution

Shazia Alam, Asma Hanif, Sumara Shaheen and Zainul Abideen: Conceptualization, Investigation, Formal analysis, Methodology. Shazia Alam, Min Zhu, Writing – original draft. Supervision, Conceptualization and review & editing Mohammad Mehdizadeh, Min Zhu and Zainul Abideen

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

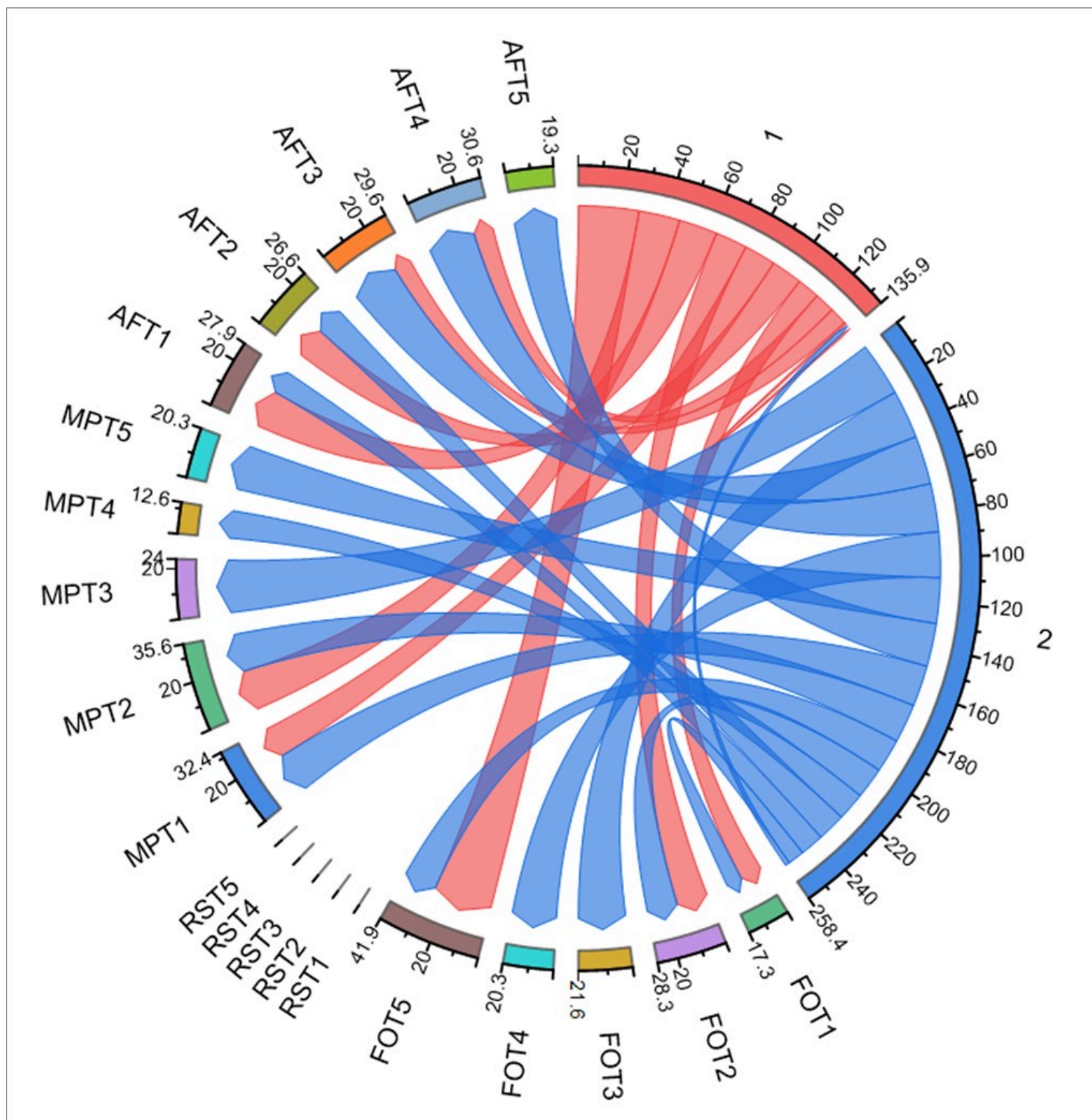


Figure 1

The Chord diagram shows the *In-vitro* anti-fungal activity of various extracts against pathogenic fungi viz., *F.oxysporum*, *R.solani*, *M.phaseolina*, and *A.flavus* using disc diffusion method. It displays the interrelation between data radially around the circle. The band width of the cord shows the significance of the data and the cords are arranged in the descending order of their weight.

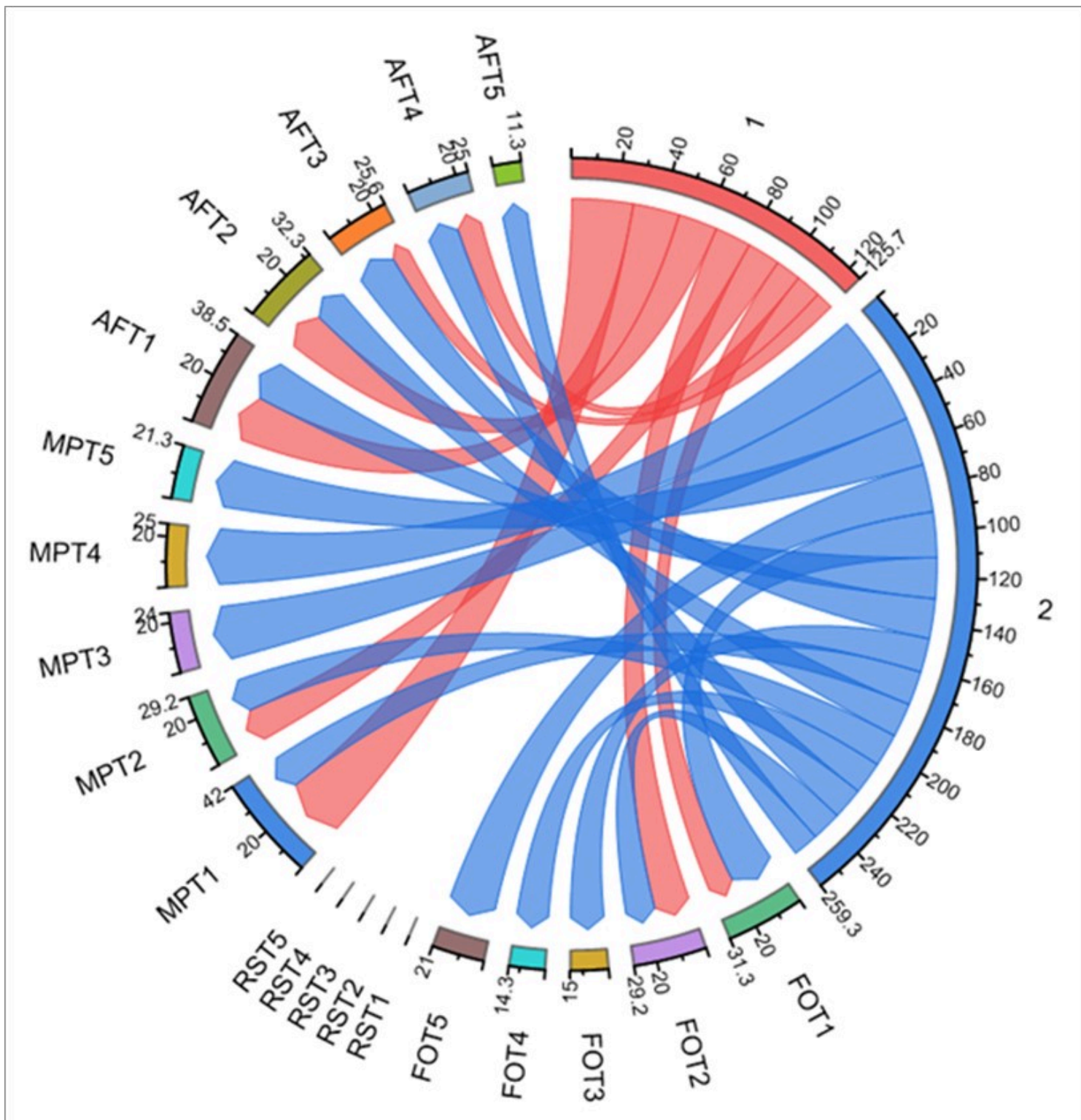


Figure 2

The Chord diagram shows the *In-vitro* anti-fungal activity of various extracts against pathogenic fungi viz., *F.oxysporum*, *R.solani*, *M.phaseolina*, and *A.flavus* using agar-well method. It displays the interrelation between data radially around the circle. The band width of the cord shows the significance of the data and the cords are arranged in the descending order of their weight.

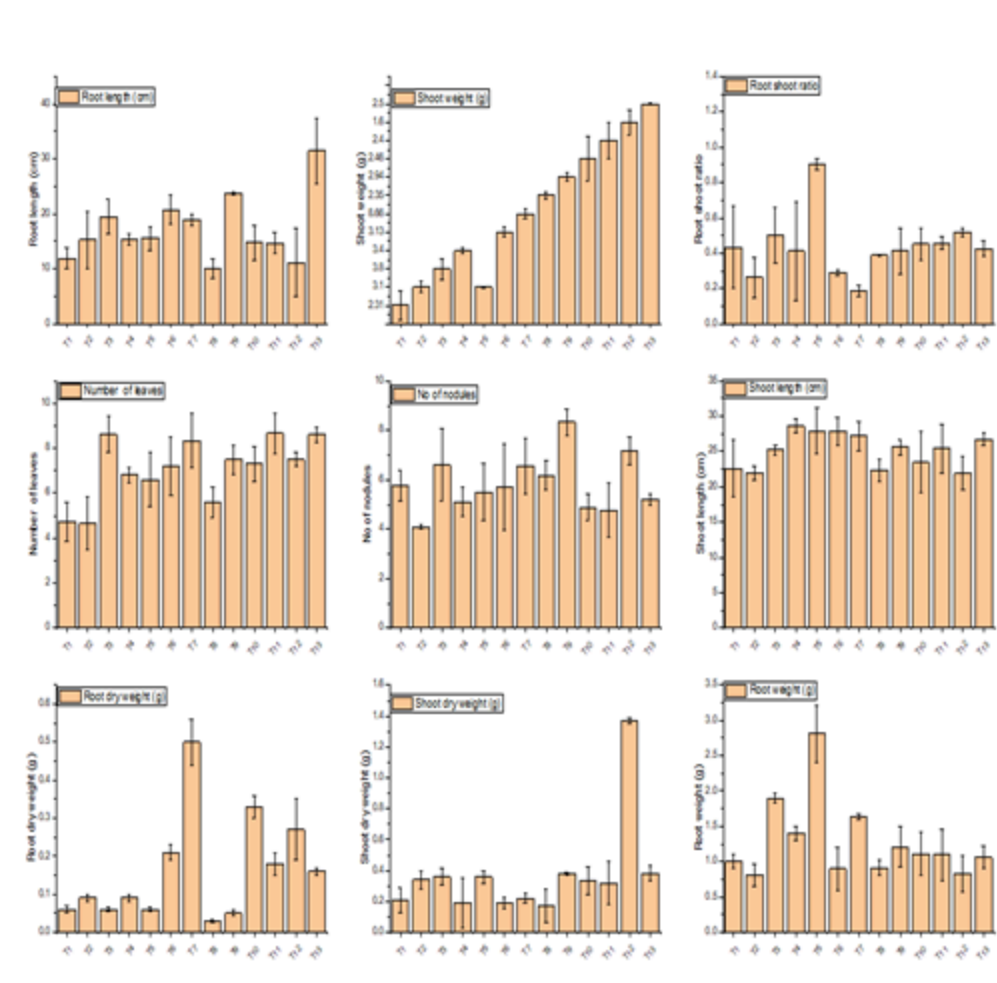


Figure 3

Application of fresh and dry ginger on the growth improvement of red beans.

Whereas, T1= control, T2=100% S.T with fresh ginger, T3=75% S.T with fresh ginger, T4=100% S.T with dry ginger, T5=75% S.T with dry ginger, T6=100% S.D with fresh ginger, T7=75% S.D with fresh ginger, T8=100% S.D with dry ginger, T9=75% S.D with dry ginger, T10=0.5% S.A with fresh ginger, T11= 0.1% S.A with fresh ginger, T12= 0.5% S.A with dry ginger, T13=0.1% S.A with dry ginger

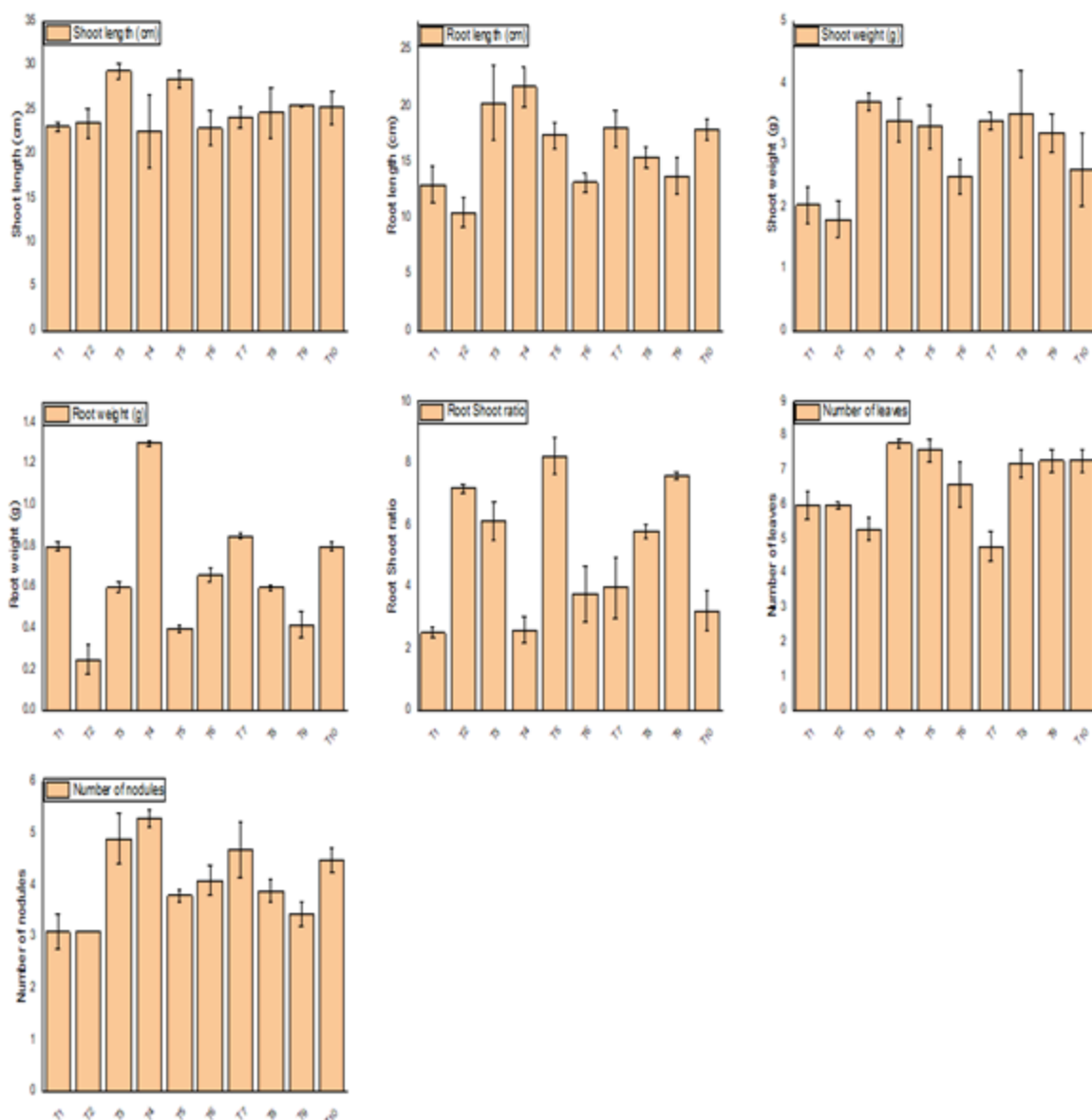


Figure 4

Effect of seed treatment by fresh ginger powder in addition with fertilizers of red bean plant on the growth. Whereas, T1=control, T2= seed treatment 100%, T3= cowdung, T4= goat dung, T5=DAP, T6=fudan, T7= cow dung +seed treatment 100%, T8= goat dung + seed treatment 100%, T9= DAP + seed treatment 100%, T10= fudan + seed treatment 100%

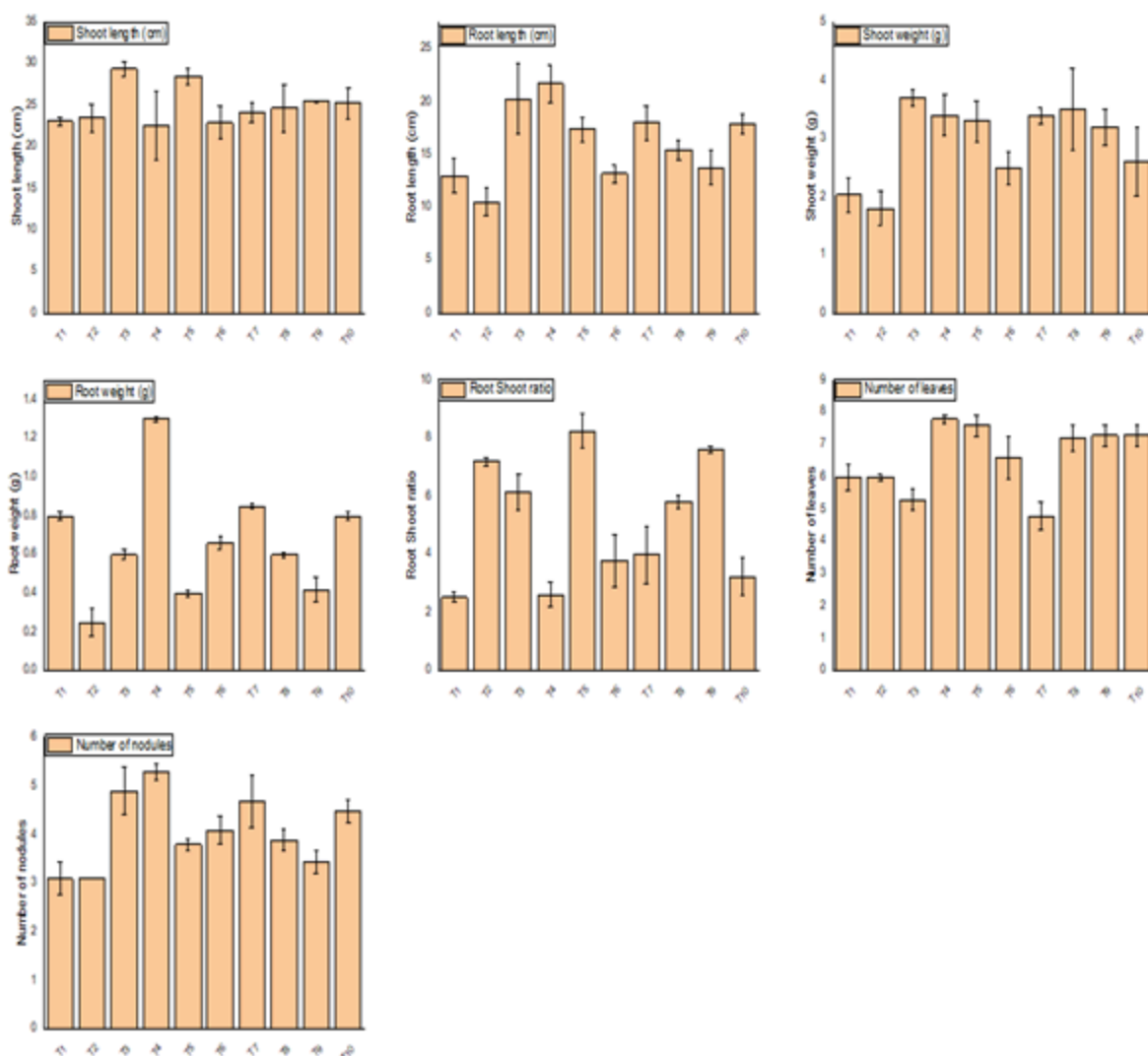


Figure 5

Effect of seed treatment by dry ginger powder in addition with fertilizers of red bean plant on the growth. Whereas, T1=control, T2= seed treatment 100%, T3= cow dung, T4= goat dung, T5=DAP, T6= fudan, T7= cowdung +seed treatment 100%, T8= goat dung + seed treatment 100%, T9= DAP + seed treatment 100%, T10= fudan + seed treatment 100%

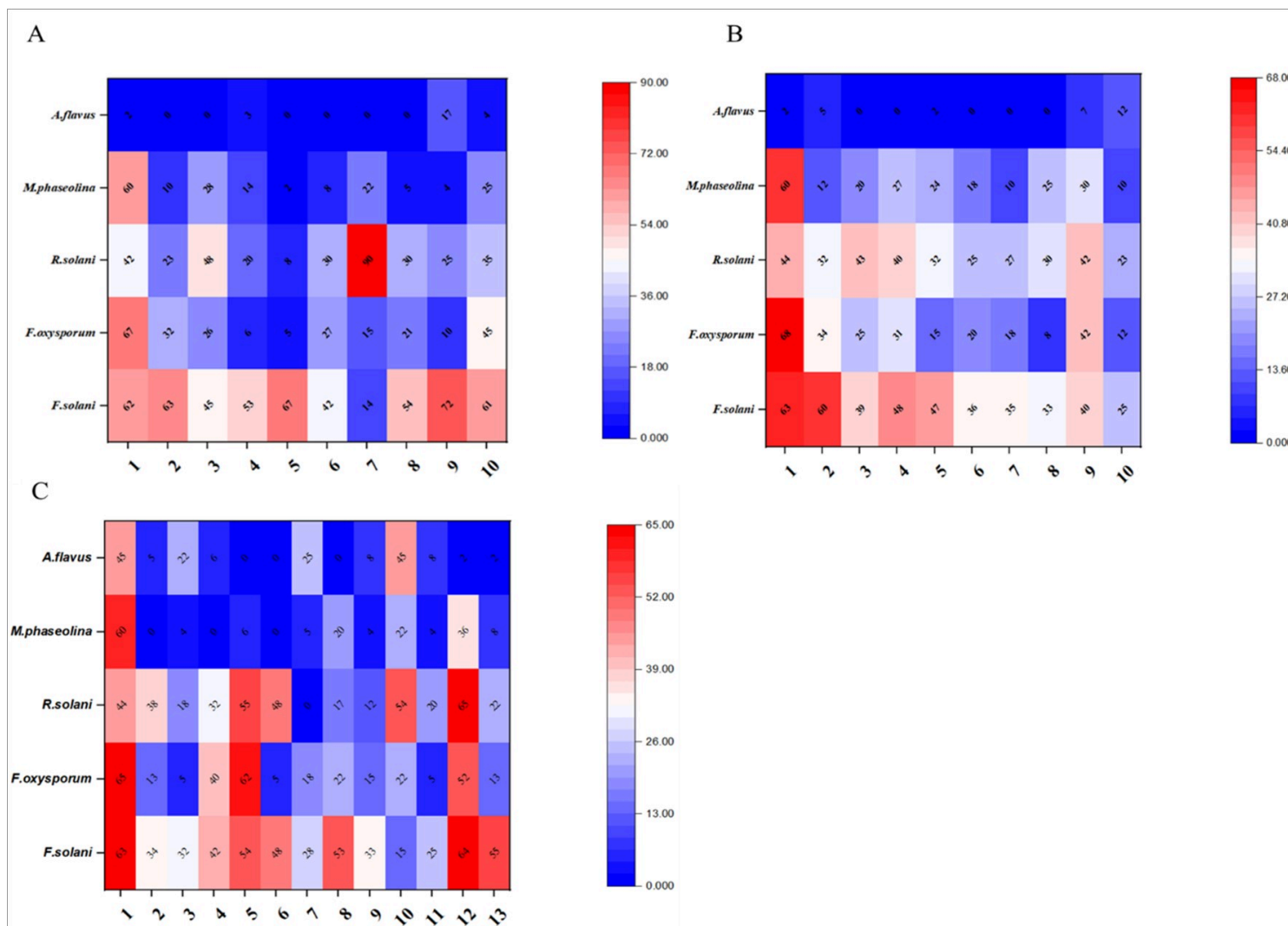


Figure 6

The heat map with labels shows the colonization percentage of *F.solani*, *F.oxysporum*, *R.solani*, *M. phaseolina*, and *A. flavus* for seed treatment with fresh ginger, dry ginger, and for soil drenching and soil amendment with fresh and dry ginger. The scale indicates the ascending order of the colonization percentages which shifts to the dark shade of red when the colonization percentage increases while the blue color indicates the minimum colonization percentage.

Whereas 6A,

1=Control, 2=Seed treatment with FG , 3=Cow dung (CD), 4=Goat dung (GD), 5=DAP, 6=Fudan (F), 7=CD+ seed treatment with FG, 8=GD + seed treatment with FG, 9=DAP + seed treatment with FG, 10=F + seed treatment with FG

Whereas 6B,

1=Control, 2=Seed treatment with DG , 3=Cow dung (CD), 4=Goat dung (GD), 5=DAP, 6=Fudan (F), 7=CD+ seed treatment with DG, 8=GD + seed treatment with DG, 9=DAP + seed treatment with DG, 10=F + seed

treatment with DG

Whereas 6C,

1=Control, 2=100% S.T with FG, 3=75% S.T with FG, 4=100%S.T with DG, 5=75%S.T with DG, 6=100% S.D with FG, 7=75% S.D with FG, 8=100%S.D with DG, 9=75%S.D with DG, 10=0.5 % S.A with FG, 11=0.1 % S.A with FG, 12=0.5 % S.A with DG, and 13=0.1 % S.A with DG.

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