ECO-FRIENDLY MANAGEMENT OF ROOT PATHOGENIC FUNGI USING HALOPHYTIC PLANTS: A STUDY ON TWO MAJOR VEGETABLE CROPS

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Abstract

Extensive exposure of fungicide and chemical fertilizers reduced crop productivity and cause degradation of ecosystem. To overcome above problem organic amendments subjugated to eliminate negative impacts of abiotic and biotic stresses which are major factors affecting crop yield. In the present study, halophytic plants *Suaeda fruticosa* (L.), and *Salsola imbricata* Forssk. leaves were utilizing to examine fungicidal effectiveness against root pathogenic fungi (*Rhizoctonia solani* Kühn, *Fusarium oxysporum* Schlect., *Macrophomina phaseolina* (Tassi) Goid). Further, plant's leaves extract and powder assessed to effect on growth of okra and cowpea crops. Significant results of improvement in growth were achieved by soil amendment with both *S. fruticosa* and *S. imbricata* leaves extract while reduction in root infecting fungi in both tested crop plants was recorded among all three methods. Soil drenching using leaves extract of both halophytic plants exhibited improved growth while soil drenching with 100% leaves extract of *S. imbricata* enhanced chlorophyll content under field condition.

KEY WORDS: *Fusarium oxysporum,* growth improvement, leaves extract, soil amendment, *Suaeda fruticosa, Salsola imbricata, Rhizoctonia solani, Macrophomina phaseolina.*

Introduction

Pesticides displayed horrible response on plants, human and their ecosystem so, scientists are attempting to find an inoffensive substance from plants parts helps not only in diminishing the frequency of infection, but also inefficacious for environment and ecosystem (Loper et al., 1991). Plants play key role as food, medicine and synthesizing chemicals. The secondary metabolites which were produce by plants are act as antimicrobial agents against the diseases of plants. Numerous plants are still under process of discovery for medicinal purposes while in Pakistan, is basic need to screen plants for their beneficial persistence (Naseem, 2012). Uptil now, 87% of advanced drugs have been isolated from plant products while many more will be isolated from plants in recent years.

Salt tolerant plants play crucial role in illustration of medicines, environmental overhauling and extensively used in field of useful drugs formation. According to researches plants parts of halophytes are used to treat diseases, however leaves considered to be more effective in comparison to other parts due to presence of phytochemicals. Phenolic compounds present in halophytic plants easily rupture fungal or bacterial cell wall ensuing cell lyses, thus exhibited strong antimicrobial, antioxidant and anticancerous activity (Skroza et al., 2019).

Salsola imbricata belongs to Chenopodiaceae family, is a native of brackish and sandy place. It contains anti-inflammatory agents, sterols, alkaloids,

phenolic compound, triterpenes, isoflavonoids and flavonoids compounds (Hamed et al., 2011; Saleem et al., 2009). However, many phytochemicals were reported from leaves extract of *S. imbricata* (Mahdavi and Mir 2006; Hamed et al., 2011; Munir et al., 2014). Another halophytic shrub, *Suaeda fruticosa* (Amaranthaceae), having ability to store and confiscate salt by evacuating glands on surface of leaves (Llanes et al., 2018; Labidi et al., 2010). Medicinally, *S. fruticosa* is helpful in curing lungs diseases, dermatitis and gastrointestinal infections. According to research, secondary metabolites present in *S. fruticosa* possesses antimicrobial, anticancer, anti-inflammatory and antioxidant properties (Shad et al., 2022; Attia-Ismail, 2016). Such information about S. *imbricata* and *S. fruticosa* will help to create a useful knowledge regarding its use to promote crop health. This study determines the application of these halophytic plants part in soil with various methods to record its effect on growth of crop plants.

Materials and methods

Plant Sample collection

Suaeda fruticosa (L.), and *Salsola imbricata* Forssk. parts (leaves, stem) were collected from University of Karachi campus and these plant samples were rinsed with tap water and then distilled water to remove the dirt. Then these plants were air dried in shade, cut into small pieces and, powdered using electrical grinder. Plant parts extract was prepared in 1:9 ratios by soaking for 24 hours in sterilized distilled water, filtered using Whattman's filter paper No.1. The resultant extract is considered as stock solution (100 % w/v concentration). Similarly, 75 and 50 % concentrations were also prepared.

Screen house Trial

Pots were filled with sandy loam soil and placed on slab of screen house at Department of Botany, University of Karachi. Each pot received 300g soil. Selected hosts were cowpea (*Vigna unguiculata* (L.) Walp cv. Lobia-2000) and okra (*Abelmoschus esculentus* (L.) Moench. var. Arka Anamika) whose seeds were sterilized in Calcium hypochlorite (1%) for 2-3 minutes, (for removing dirt particles) and wash twice with sterilized distilled water. Sterilized seeds of okra and cowpea were treated with leaves extract of *S. imbricata* and *S. fruticosa* separately for 5-10 minutes and air dried in the laminar flow chamber. Different methods of application were included soil amendment with 3 different concentrations (0.1, 0.5, 1%w/w), seed treatment (50, 75 and 100% w/v) and soil drenching (50, 75 and 100 % w/v) with leaves extracts of *S. imbricata* and *S. fruticosa*, respectively. The pots labeled for soil drenching receives 10 mL of all concentrations in each pot separately. For soil amendment, soil was mixed with leaves powder of *S. imbricata* and *S. fruticosa* (0.1, 0.5 and 01% w/w, respectively) while for seed treatment, treated seeds with 50, 75 and 100 % w/v concentrations of leaves extracts of *S. imbricata* and *S. fruticosa* was sown in pots, respectively. Control pots containing untreated seeds or drenched with distilled water was also set for comparison. There were three replicates for each treatment. Pots were daily sprinkled with adequate amount of water until uprooting. After 30 days of germination, pots were taken to laboratory for observation of growth parameters and root colonization by root pathogenic fungi.

Soil Analysis

Experimental soil was analyzed for soil pH and electrical conductivity following by the method of Brady (1990) and Sparks (1996). Root infecting fungi isolated and identified by the method of Ejaz (2025).

Field Trial

Microplots with surface area 729 cm^2 were prepared in randomized complete block manner at the field of the Department of Botany, University of Karachi. Population of root infecting fungi (*Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina*) were determined (Wilhelm, 1955; Nash & Snyder, 1962; Sheikh & Ghaffar, 1975). Treatments include seed treatment and soil drenching with 100% leaves extract of *S. imbricata* and *S. fruticosa*. These treatments were selected for field trial as these treatments showed most promising results in pot trial. Experiment was terminated after 90 days of germination. Growth indices and colonization of roots by pathogenic fungi were recorded.

Photosynthetic pigment analysis

Estimation of Chlorophyll a, b, chlorophyll ratio and carotenoids were observed using the method of Duxbury and Yentsh (1956). Fresh and young leaves (1g) of each treatment was crushed with 80% acetone and centrifuged (3000rmp for 15 minutes). Supernatant was collected and optical density was recorded at 663, 645 and 652 using spectrophotometer.

The formulas for calculation are as follows:

$$\text{Chlorophyll a} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{\text{Final volume of supernatant} \times 1000}{\text{Fresh weight of sample}}$$

$$\text{Chlorophyll b} = 22.9(A_{645}) - 4.69(A_{663}) \times \frac{\text{Final volume of supernatant} \times 1000}{\text{Fresh weight of sample}}$$

$$\text{Total Chlorophyll} = \frac{(\text{absorbance at 652nm})}{34.5} \times \frac{1000 \times \text{Final volume of supernatant (5mL)}}{1000 \times \text{Fresh weight of sample (1g)}}$$

$$\text{Chlorophyll ratio} = \text{Chlorophyll a} / \text{Chlorophyll b}$$

Data Analysis

One- or two-way analysis was used for analyzing the data obtained. However, Duncan's multiple range test (DMRT) at 5 % level was used to compare means (Zar, 2009; Gomez & Gomez, 1984). All means were displayed as mean ± standard error (SE).

Results

Effect of halophytic plant on soil EC and pH

Increased EC was recorded with respect to control after 30 days of germination of both crop plants in seed treatment, soil drenching and soil amendment of *S. fruticosa* leaves extract and powder in cowpea and okra whereas, *S. imbricata* showed ineffectiveness in cowpea with respect to soil conductivity. On the other hand, similar efficacy was exhibited for okra significantly which indicated that halophytic plants increased

electrical conductivity of soil as the amount of concentrations of salt were increased. On the contrary, minimal differences in pH were observed that is alkaline pH in all three concentrations with respect to control in both crop plants (Figure 1).

Screen house trial

Maximum germination of cowpea was observed in seed treatment and soil drenching with *S. imbricata* in almost all concentrations (P< 0.001). Significant (P< 0.001) enhancement in growth indices like shoot length, shoot weight and root weight were recorded due to soil amendment with *S. imbricata* at 0.1% w/w concentration followed by soil drenching with *S. imbricata* at 75 and 100% w/v concentrations (Figure 2). Improvement in root length was achieved when soil was amended with *S. fruticosa* at 0.1 w/w concentration followed by seed treatment with *S. imbricata* at 50% w/v concentration (P< 0.001). It was noted that all methods gave significant (P<0.001) reduction in root colonizing fungi in contrast to control. However, soil drenching with 50 and 100 % w/v concentration of *S. fruticosa* gave complete reduction of *R. solani*, *M. phaseolina*, and *F. oxysporum* (P< 0.001). Seed treatment with 75% w/v, soil amendment with 1% w/w and soil drenching with 75% w/v of *S. fruticosa* also showed reduced colonization of root infecting in roots of cowpea plant (Figure 2).

Germination of okra plant was maximum with soil amendment with 0.1% w/w concentration of *S. fruticosa* followed by 0.5% w/w concentrations of *S. fruticosa* and *S. imbricata* (P< 0.01). Significant root length (P< 0.001) was achieved when soil was amended by 0.5 % w/w concentration of both halophytic plants. Seed treatment with 100% w/v of *S. fruticosa* showed improved shoot length (P< 0.001) while shoot weight was higher when soil was amended at 0.1% w/w concentration of *S. fruticosa.* Root weight was remarkably gained when soil was amended with 0.5% w/w concentration of *S. imbricata* followed by 100% w/v seed treatment with *S. fruticosa.* Seed treatment with *S. imbricata* at all concentrations displayed suppression of *F. oxysporum* and *R. solani* colonization compared to control plants. However, soil drenching with 50% w/v concentrations of both halophytic plants completely reduced colonization of *M. phaseolina*, *R. solani* and *F. oxysporum* (P< 0.01; Figure 3).

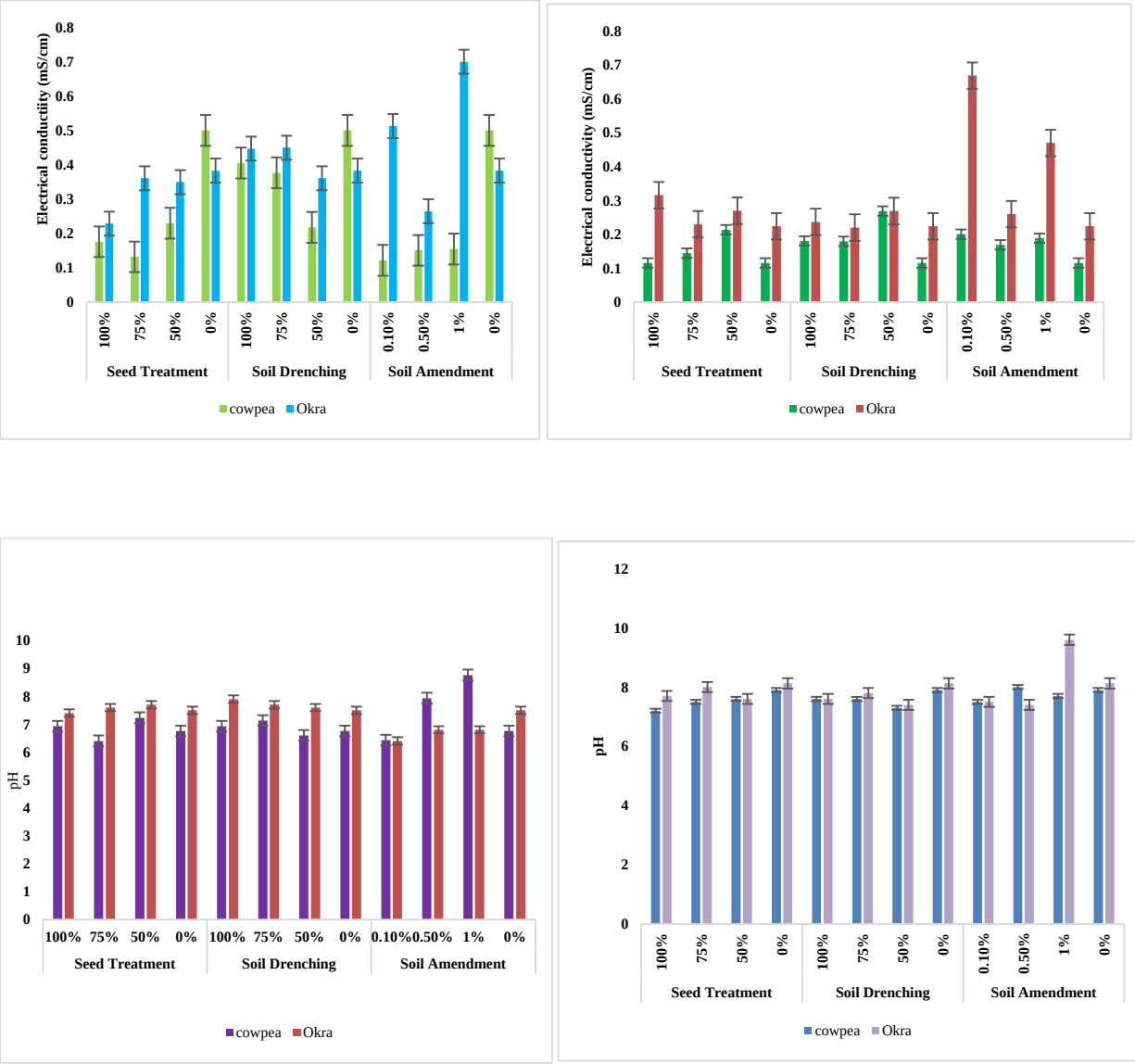


Figure 1: Effect of *S. imbricata* and *S. fruticosa* leaves extract and powder on soil electrical conductivity and pH. (Electrical conductivity of distilled water is 0.02 (mS/cm) and pH is 07). Each bar represents mean of 3 replicates ± standard error (SE).

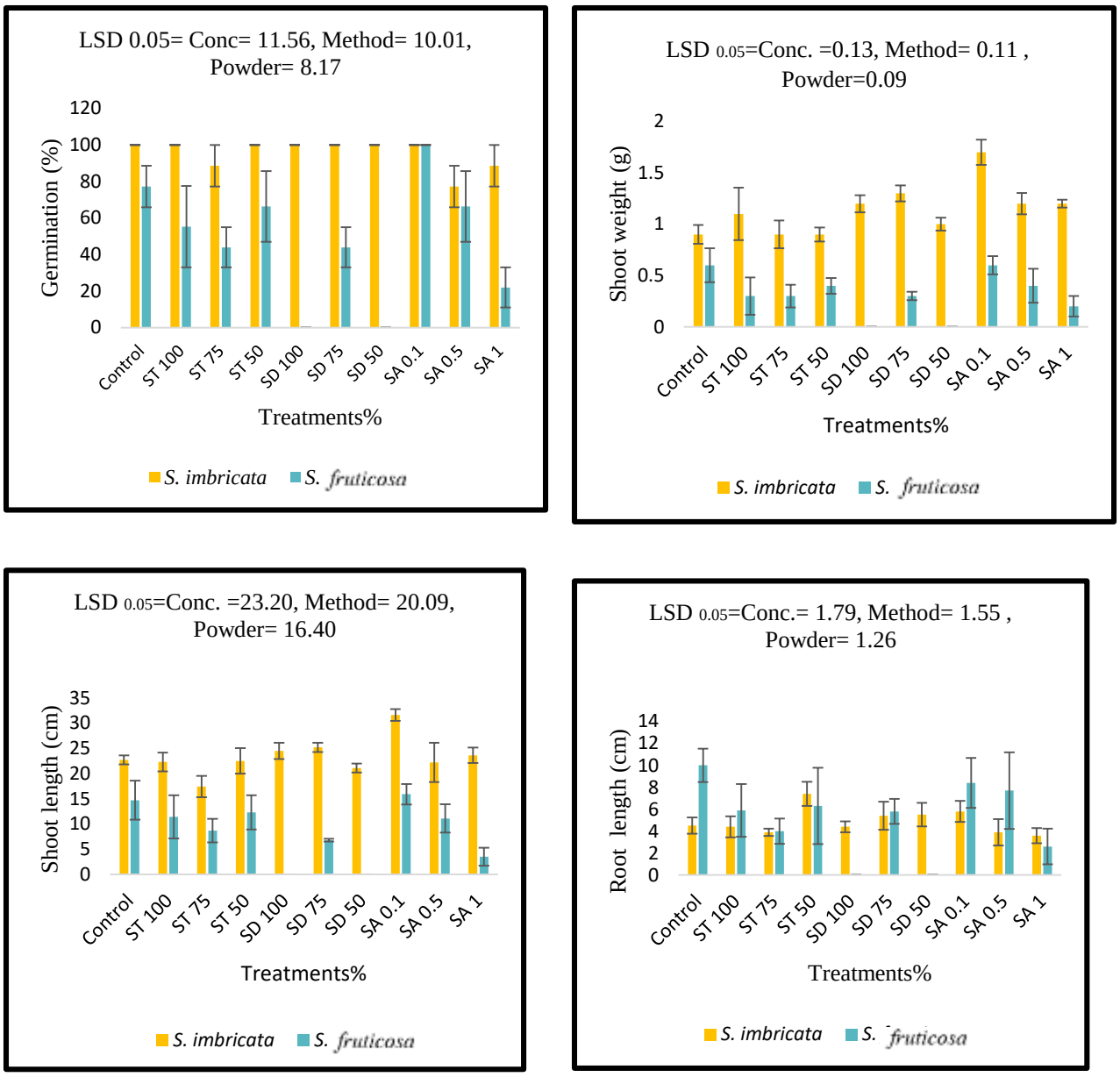
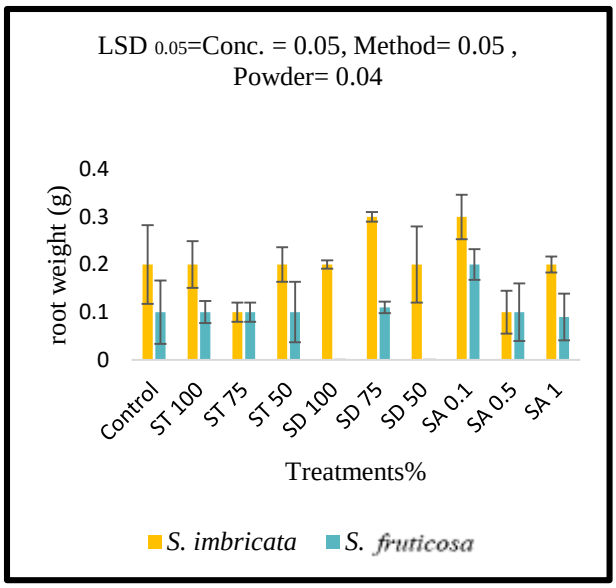
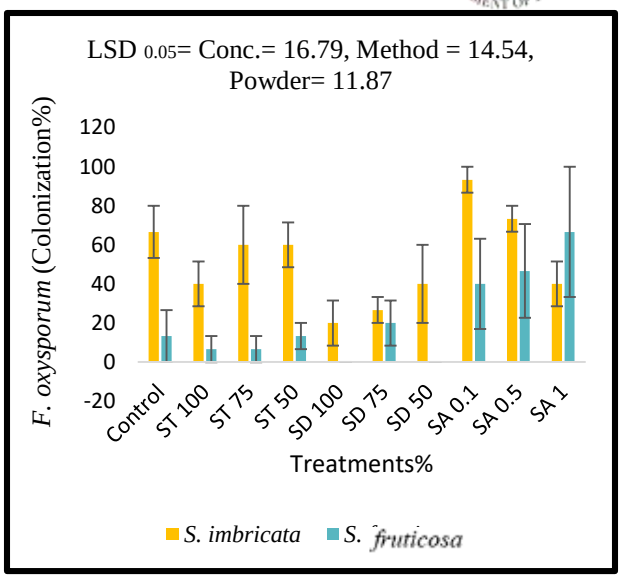
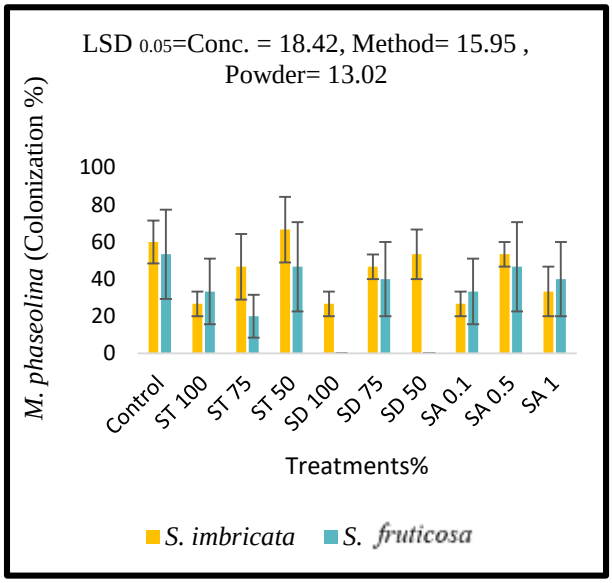


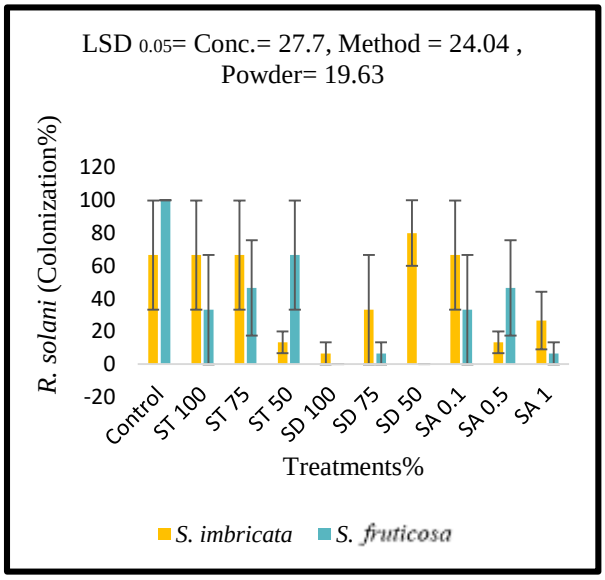
Figure.2. Impact of *S. imbricata* and *S. fruticosa* leaves extract and powder on cowpea plant.

ST= Seed Treatment, SD= Soil Drenching, SA= Soil Amendment. Each bar represents mean of 3 replicates ± standard error (SE).


LSD 0.05=Conc. = 0.05, Method= 0.05 , Powder= 0.04
root weight (g)
0.4
0.3
0.2
0.1
0
Control
ST 100
ST 75
ST 50
SD 100
SD 75
SD 50
SA 0.1
SA 0.5
SA 1
Treatments%
S. imbricata
S. fruticosa


LSD 0.05= Conc.= 16.79, Method = 14.54, Powder= 11.87
F. oxysporum (Colonization%)
120
100
80
60
40
20
0
-20
Control
ST 100
ST 75
ST 50
SD 100
SD 75
SD 50
SA 0.1
SA 0.5
SA 1
Treatments%
S. imbricata
S. fruticosa


LSD 0.05=Conc. = 18.42, Method= 15.95 , Powder= 13.02
M. phaseolina (Colonization %)
100
80
60
40
20
0
Control
ST 100
ST 75
ST 50
SD 100
SD 75
SD 50
SA 0.1
SA 0.5
SA 1
Treatments%
S. imbricata
S. fruticosa


LSD 0.05= Conc.= 27.7, Method = 24.04 , Powder= 19.63
R. solani (Colonization%)
120
100
80
60
40
20
0
-20
Control
ST 100
ST 75
ST 50
SD 100
SD 75
SD 50
SA 0.1
SA 0.5
SA 1
Treatments%
S. imbricata
S. fruticosa

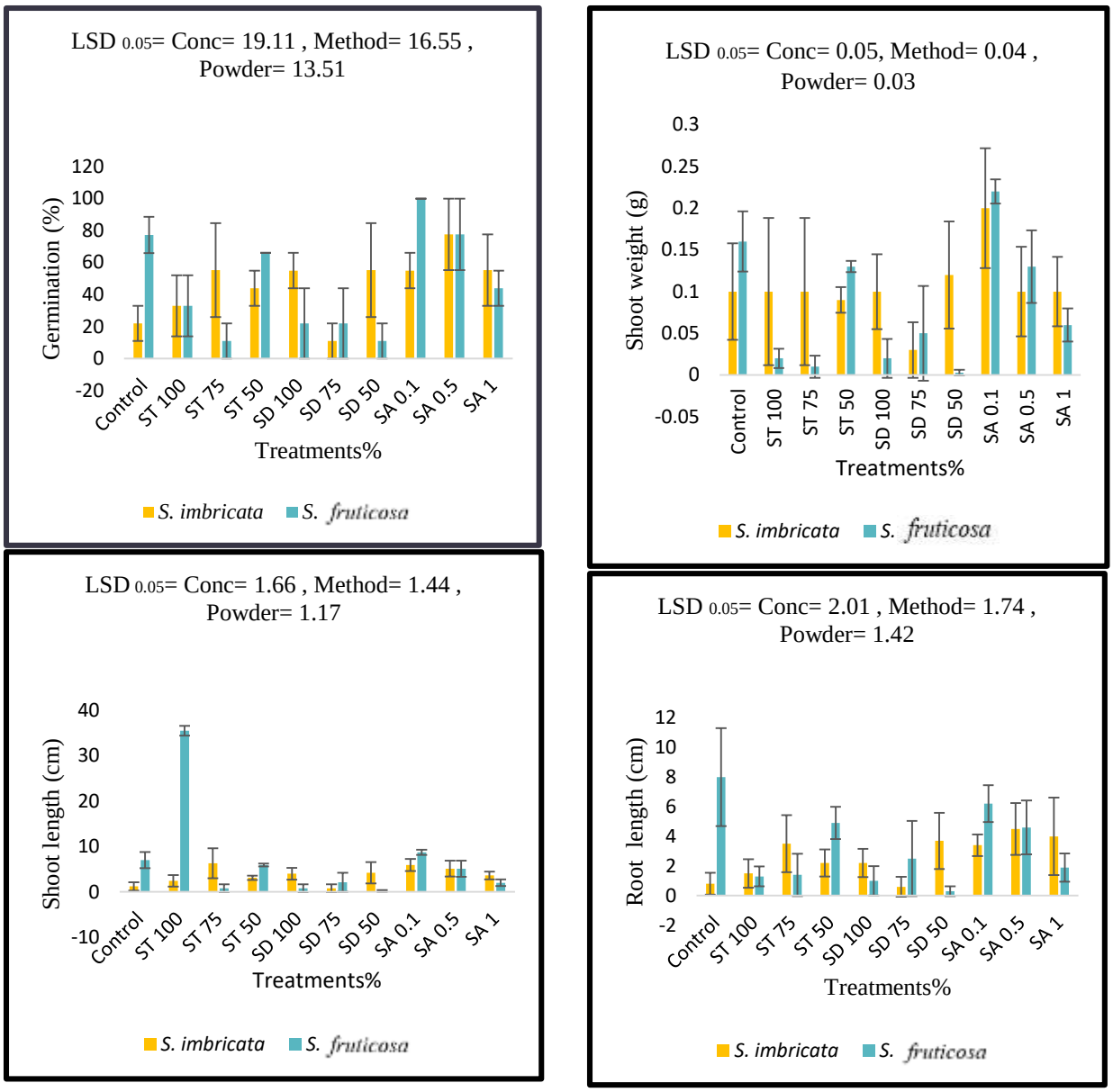
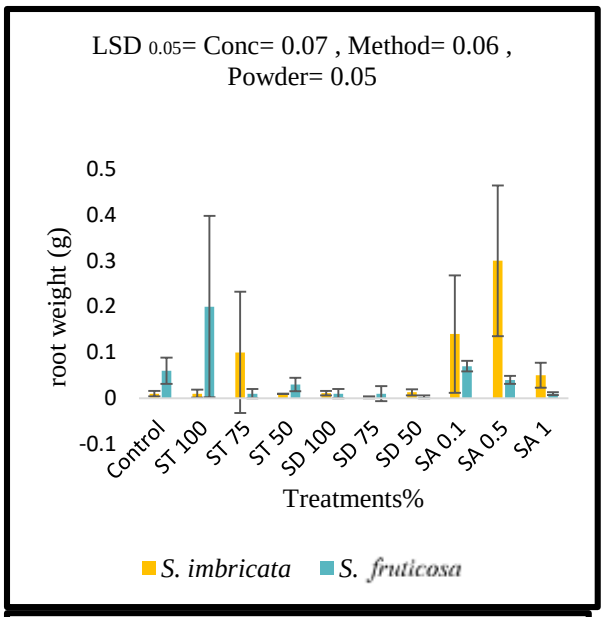
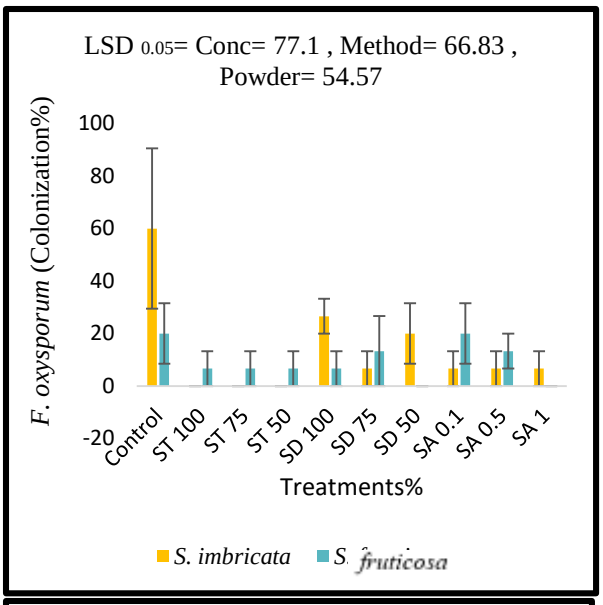
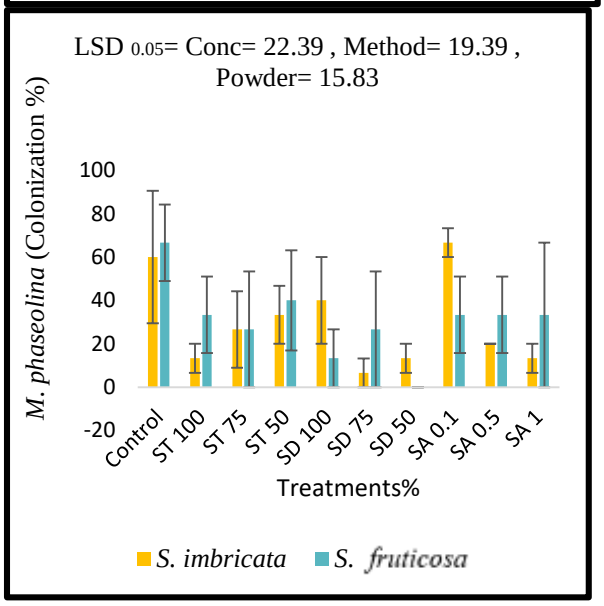


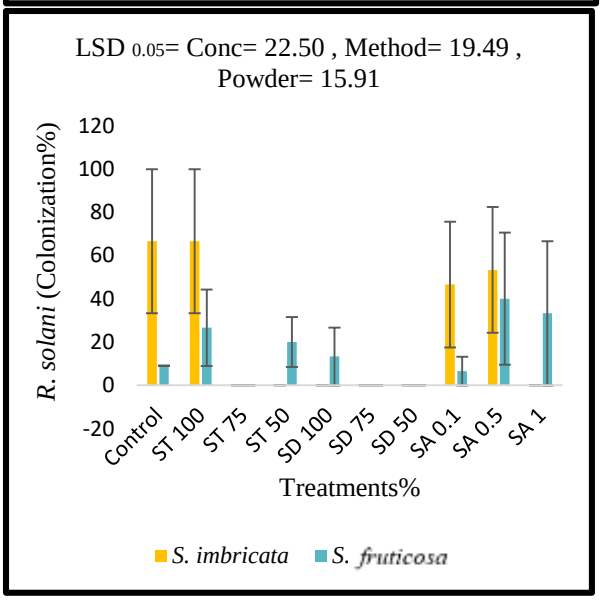
Figure.3. Impact of *S. imbricata* and *S. fruticosa* leaves extract and powder on okra plant.

ST= Seed Treatment, SD= Soil Drenching, SA= Soil Amendment. Each bar represents mean of 3 replicates ± standard error (SE).





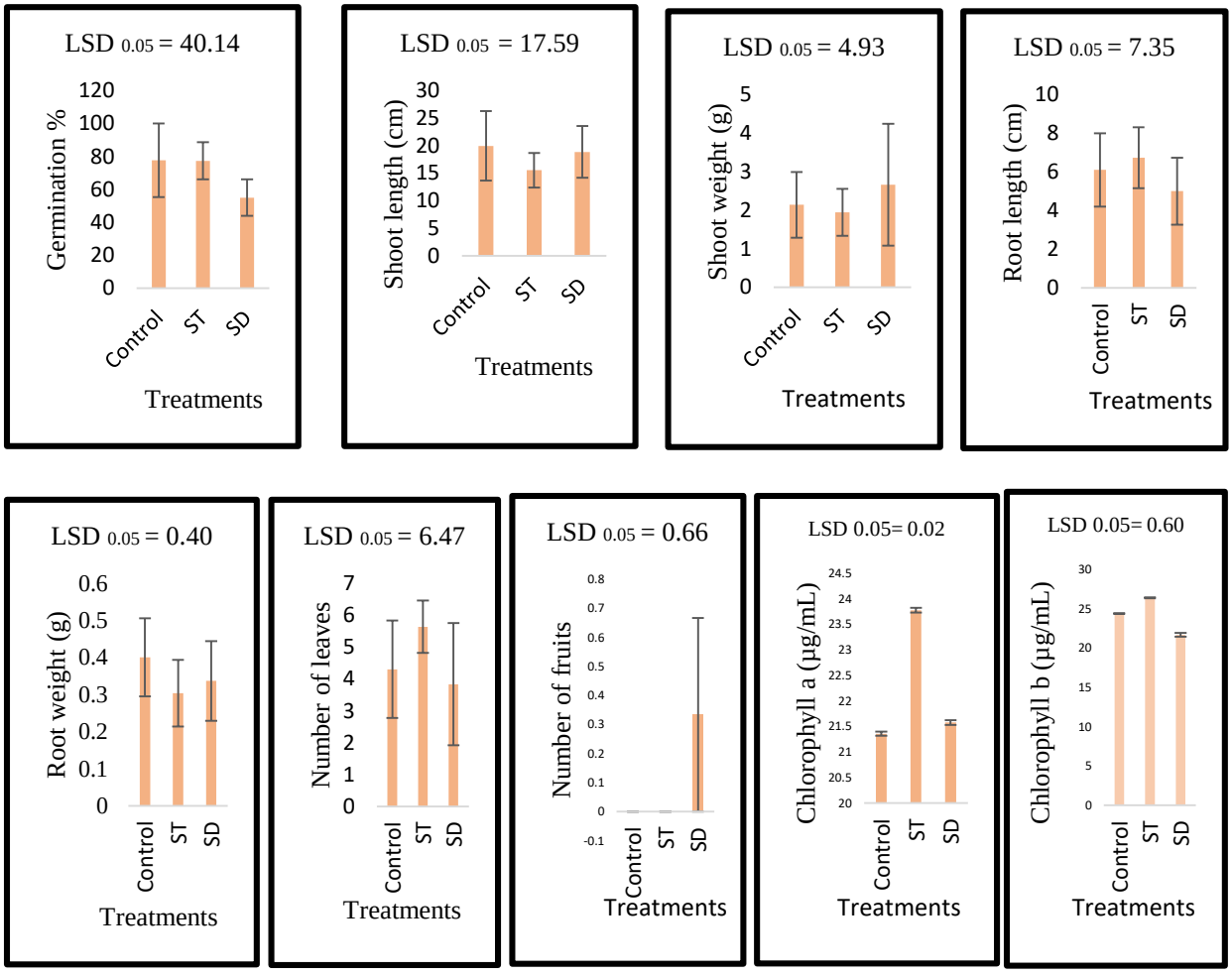




Field Trial

Under field conditions, *S. imbricata* improved germination remarkably while soil drenching with 100 % w/v leaves extract of *S. imbricata* enhance the shoot length and weight of cowpea plant. However, root length and number of leaves of cowpea were maximum when seeds were treated with *S. imbricata* while number of fruits were strengthened by using soil drenching method as compared to control. Photosynthetic pigments like chlorophyll a, chlorophyll b and total chlorophyll significantly ($P<0.001$) improved due to seed treatment with *S. imbricata* extract at 100% concentration while chlorophyll ratio was improved when *S. imbricata* extract was drenched n soil ($P<0.001$). Colonization with root pathogenic fungi exhibited remarkable decline ($P<0.05$) in both methods while *R. solani* was completely inhibited in treated seeds (Figure. 4).

In case of okra plant, soil drenching with 100% extract of *S. imbricata* leaves exhibited 100% germination, improved growth indices like shoot length, shoot weight, root length, root weight, number of leaves and number of fruits. However, chlorophyll a, b, total chlorophyll and its ratio was enhanced in okra plant due to soil drenching with 100% extract of *S. imbricata* remarkably (P<0.001) while seed treated method reduced chlorophyll content in comparison to control. Root colonization by pathogenic fungi like *M. phaseolina* was entirely controlled by soil drenching method (P<0.01) while *F. oxysporum* colonization was reduced significantly (P<0.05) by seed treatment. However, *R. solani* was significantly inhibited (P<0.05) in seed treated with *S. imbricata* leaves extract (Figure 5).



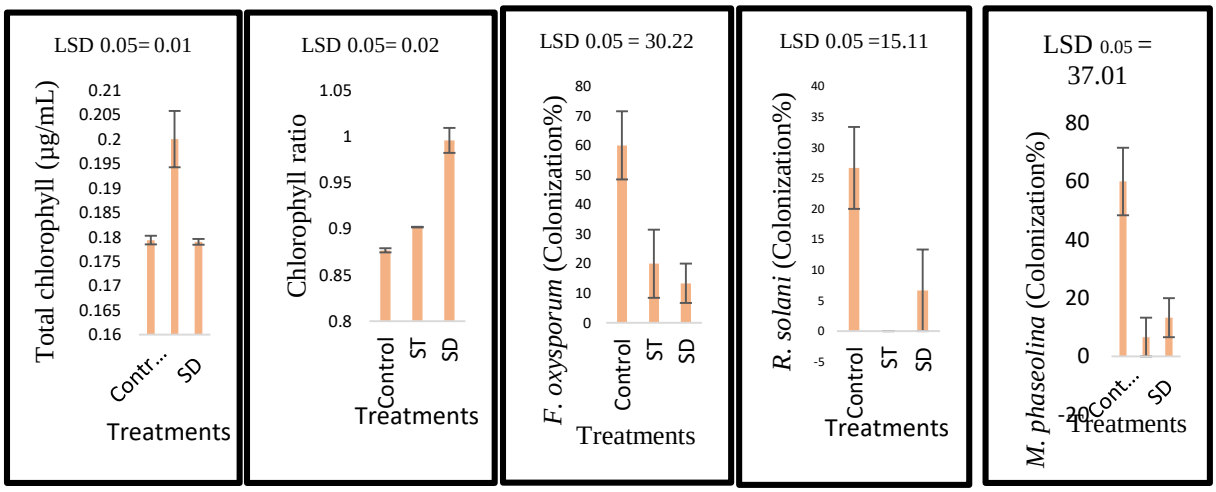
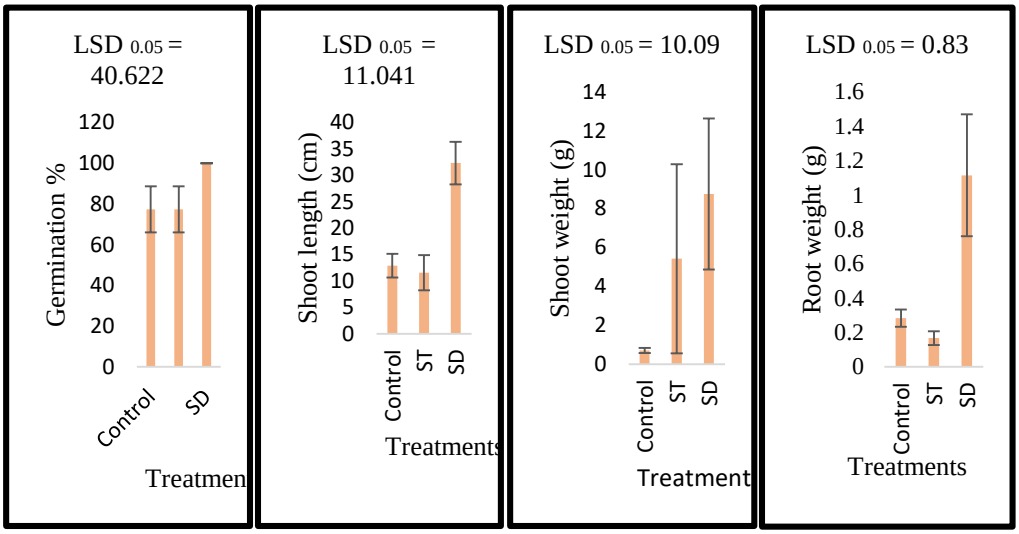


Figure.4. Influence of *S. imbricata* leaves extract on cowpea plant. (ST= Seed Treatment and SD= Soil Drenching). Each bar represents mean of 3 replicates ± standard error (SE).



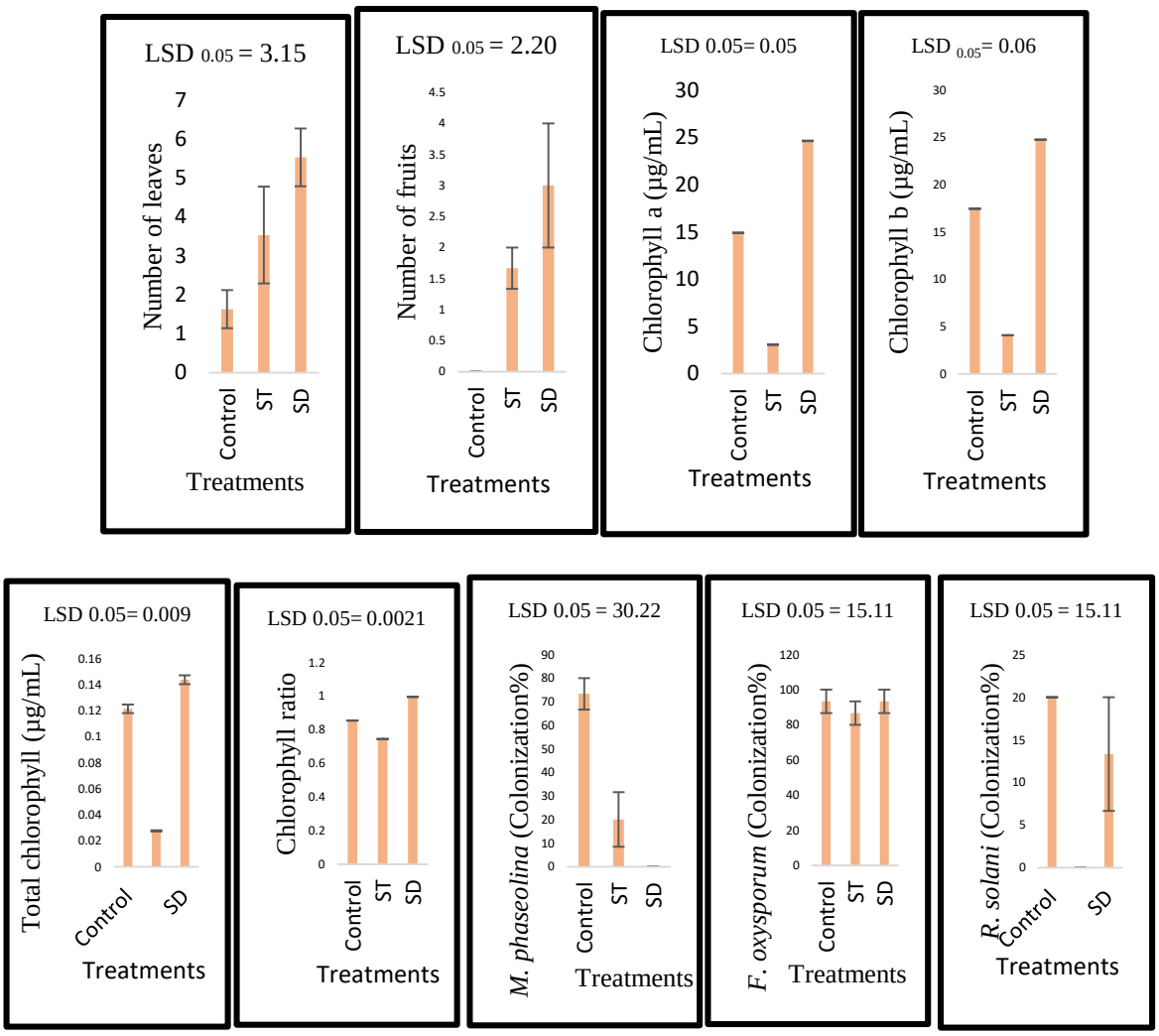


Figure.5. Influence of *S. imbricata* leaves extract on okra plant (ST= Seed Treatment and SD= Soil Drenching). Each bar represents mean of 3 replicates ± standard error (SE).

Discussion

Halophytes are considered to be the plants of salt marches and unfertile land that can sustain themselves under extreme environmental circumstances and unendurable weather stress due to its adaptable features. It plays fundamental role in ecosystem in form of biofuel, food, fodder etc. and utilizes in pharmaceutics, neutraceutics and agricultural practices (Roy et al., 2018; Flower & Colmer,2008). Many species of halophytes like *Suaeda, Distichlis, Salicornia* and *Atriplex* are frequently used of its cellulosic constituent which is used to produce biofuel (Rozema et al., 2008; Xian-Zhao et al., 2012). Astonishingly they are believed to be extremophiles due to its tolerance towards extreme saline condition (Kosová et al., 2013). In accordance of researches, dicot halophytes are more prone to endure salt concentration as compared to monocot halophytes (Flower & Colmer, 2008). Researches reflect that many halophytic plants have been used against bacterial and fungal infections such as skin disorders caused by *candida* and oral infections by *Streptococcus*. It also has been used to recover wound externally on insect bite as

well as in order to cure gastrointestinal tract disorders (Geraghty et al., 2011).

Antifungal activity of *S. imbricata* and *S. fruticose* leaves extract were examined against root pathogenic fungi on two major crop plants (Cowpea and okra) in present studies. Both halophytic treatments successfully repressed root pathogenic fungi in addition with amelioration of growth parameters with approximately all three methods (Seed treatment, Soil drenching and soil amendment). 100 and 75% w/v concentrations proved to be more effective in seed treated and soil drenched methods on the other hand, 0.1 and 0.5% w/w concentrations were reflected to be more successful in soil amendment method resulting decline in root rot infection and increase in growth parameters remarkably. Extract based biofungicides brings important benefits to farmers in case of food security, reduction in phytopathogens, improvement in quality of crops (Tripathi, et al., 2020). It is important to note that plant based chemical compounds are not always specific to target organisms but are non-toxic pollination, predatory bees and climate (Al-Mekhlafi, et al., 2023). Our present result was in confirmation with Hernandez Soto et al (2018) which showed that 60% of growth inhibition was recorded with *Argemone ochroleuca* leaves against *Fusarium oxysporum* due to presence of compounds like, isoquinoine, ehydrocorydalmin and oxyberine. Similarly, *Thymus vulgaris* and *Zingiber officinale* were highly active against *phytopathogenic* fungi like *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani* (Al-Rahmah et al., 2013).

Chemical profiling of root and shoot extracts of *Suaeda tetragona* reported to have high amount of phenolics and nitrogenous compounds, *Suaeda tetrenda* and *S. vermiculata contain phenolic acids and saponins which* make them anti-inflammatory. Shoot extract showed more efficacy as compared to root extract and the ability of anti-inflammatory response of these species were higher than that of usual drug piroxicam (Zakaria et al., 2025). Al-Thohamy et al., (2018) reported that fat constituent of *Salsola vermiculata* and *Suaeda vermiculata* make them effective against many bacterial species while they are rich in alkaloids, flavonoids and antioxidant compounds which make them antimicrobial in nature. Major focus of scientist in the field of research is to maximize use of natural product in comparison to xenobiotic chemicals against plant pathogen (Kiran et al. 2006).

Conclusion:

Synthetic fungicides produce harsh impact on environment and health. This will increase the demand for plant-based products. These products are effective, cheap and harmless to soil and environment. Different formulations prepared from *Suaeda fruticosa* (L.), and *Salsola imbricata* Forssk. leaves were applied to cowpea and okra plant to record its effect on growth and reduction in colonization of pathogenic root fungi. From the three methods, soil amendment with both halophytic plants showed improvement in growth of tested while soil drenching at 100 % w/v gave effective result in suppression of root pathogenic fungi. These plants based organic fungicides plays a crucial role in improving the productivity of crops with minimizing environmental impact by prioritizing sustainable agricultural practices.

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