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ENCAPSULATION OF MEDICINAL SEEDS POWDER TO CONTROL ROOT PATHOGENIC FUNGI BY AMELIORATING THE PHYSIOLOGY AND GROWTH OF OIL YIELDING CROPS

KAMRAN ASHRAF[1], ASMA HANIF[1], SHAHNAZ DAWAR[1], MUHAMMAD UMAR[2] AND ZAMIN SHAHEED SIDDIQUI[2]

[1]Department of Botany, University of Karachi, Karachi-75270, Pakistan
[2]Stress PhysiologyPhenomic Centre, Department of Botany, University of Karachi, Pakistan
**Corresponding author's email: asma.hanif@uok.edu.pk/shahnaz_dawar@yahoo.com*

Abstract

Oil yielding crops have incredible significance which influences the agribusiness of Pakistan. Present research was designed to investigate the encapsulation of medicinal seeds powder in the management of root deteriorating fungi. Ethanolic extract of seed powders were obtained from twenty plant species and was used at 10,000ppm and 5,000ppm, while DMSO (Dimethyl sulfoxide) and Metiram (fungicide) taken as control to check the antifungal activity *In vitro* against root pathogenic fungi (*Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina*). Among the tested seeds, *Acacia nilotica* seed powder showed excellent inhibition of fungal pathogens at 10,000ppm in paper disc and well methods and was selected *In vivo* experiment. Capsules of *Acacia nilotica* seeds powder was amended in soil not only improved the growth of oil yielding crops but also increased the photosynthetic process and chlorophyll content.

Key words: Capsules, Medicinal seeds powder, Root decay pathogens andoil crops.

Introduction

Root pathogens caused 26% loss of agrarian yields (Kumar, 2016). Plant pathogenic fungi are ubiquitous and considered as perilous to crop production that cause substantial monetary losses (Ojaghian *et al.*, 2016). *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina* are root rot pathogens which causes vascular blockage in roots leading to the death of plants (Gamliel *et al.*, 2000). *R. solani* is a necrotrophic fungus and considered as root/hypocotyl pathogen (Guerrero-González *et al.*, 2011). *M. phaseolina* is a pathogenic fungus that survives in a soil for long period (≥15 years) with their sclerotia/chlamydospores (Sanchez *et al.*, 2016) and infected plant contains charcoal rot disease due to sclerotia development in the plant crown (Gaige *et al.*, 2010). *Fusarium* spp. considered as one of the notorious, abundant, prevalent and imperative fungi produces damping off seedling, vascular wilts and rots of stem/root diseases in plants (Cha *et al*., 2016).

Amongst the latest biological approaches to root protection, pathologist recommended that organic amendments applied to field not only improve the soil but also stimulate the natural defense mechanism in plantsagainst plant pathogens (Walters & Fountaine, 2009). Researchers reported that the application of medicinal plant seeds powder amended in soil at low concentration released phenols, organic acids, tannins and nitrogenous compounds to overcome root diseases and increases agricultural productivity (Ahmed *et al*., 2009; Adeleke *et al*., 2017). Medicinal seeds powder when amended in the soil can stop the reproduction of pathogenic fungi which sound to be ecologically safe strategies for crop defense (Lee *et al.,* 2007). Medicinal plant seeds possess a good rank among other seed because of 75% oil stored in cotyledons tissues (Singh *et al*., 2011). Fiber present in it which have several benefits due to the presence of large amount of Alpha-Linolenic Acid (Rabetafika *et al*., 2011). Cotyledons contains about 21% of protein, main proteins are albumin 20-42% and globulin 26-58% which improve the antioxidants level present in the seeds (Oomah, 2001). Medicinal plant seeds contain essential oils and chemical compounds like terpenoids and non-terpenoids which exhibit allelopathic properties, notably very small amount of data is present about the allelochemical activities of seeds (Isman, 2000). Using seeds extract for controlling plant pathogens would decrease the death of plants and lower the amount of money spent on agrochemicals but also enhance the palatability of the fruits (Fawzi *et al*., 2009).

In Pakistan, oil seed crops cultivated about 0.596 million ha (Amjad, 2014) but unfortunately agriculturist was unable to fulfill the requirement of edible oil consumption due to increasing of urban population, eating habit of city dwellers, storage issue, mismanagement of heavy used of agrochemicals, lack of finance, technology and entrepreneurship. Knowledge on the application of medicinal plant parts especially plant seeds are not researched properly, it will be vanished from next generations if not giving its importance (Hostettmann *et al*., 2000). Indigenous plant seeds are not tested mostly and still much study is required in this regard; therefore, more investigation is recommended to explore the fungicidal potential of seeds.

Keeping in view, present research work was based on the screening of some organic medicinal seeds against root pathogenic fungi using encapsulated organic seed powder, amended in the soil to study the connection between the growth of oil crops and its effect on the colonization of pathogenic fungi.

Materials and Methods

1. Collection of organic medicinal seeds and oil seed crops: Indigenous seeds include *Acacia nilotica* (L.) Delile, *Adenanther apavonina* (L.), *Albizia lebbeck* (L.)

Benth., *Cassia fistula* (L.), *Conocarpus erectus* (L.), *Dalbergias issoo*Roxb., *Delonix regia* (Boj. ex Hook.) Raf., *Ficus religiosa* (L.), *Leucaena leucocephala* (Lam.) de Wit, *Linum usitatissimum* (L.), *Moringa oleifera* Lam., *Parkinsonia aculeate* (L.), *Phoenix dactylifera* (L.), *Prosopis juliflora* (Sw.) DC., *Salvia hispanica* (L.), *Sapindus mukorossi* (Gaertn.), *Tamarindus indica* (L.), *Thespesia populnea* (L.) Sol. ex Correa, *Terminalia catappa* (L.) and *Withania somnifera* (L.) Dunal were collected from different places in Karachi. Seeds of corn/maize (*Zea mays* L. variety- GOLDEN), peanut/groundnut (*Arachishypogaea*L. variety- Desi #334) and sunflower (*Helianthus annuus* L. variety-Hysun #38) were purchased from the confined market of New town (Purani sabzimandi), Karachi. Oil seeds were surface sterilized with 1.0% sodium hypochlorite for five minutes followed by thrice washings with sterile water for 3-5 minutes before sowing (Ervin & Wetzel, 2002).

***In vitro* experiments:** Organic medicinal seeds were powdered respectively and each powdered (20g) was soaked in 100mL of methanol, plugged with cotton and kept on a rotary shaker for 48 hours of duration for complete extraction of chemicals. The extracts were filtered using Whatman No.1 filter paper and the crude methanolic extracts were evaporated to dryness at room temperature (27-30°C) for few days for the complete evaporation of methanol to obtain gummy extract (Saadeghi-Nejad and Azish, 2013). Dried extracts were stored in labeled sterile screw capped bottles for further work. 0.1g of gummy extract was dissolved in 10mL of DMSO (Dimethyl sulfoxide) in sterile labeled capped vials by which 10,000 ppm solution obtained, while 5000 ppm was made by using 5mL of DMSO and 5mL of 10,000 ppm to study the zone of growth inhibition of root rot fungal pathogens (Nkere & Iroegbu, 2005) using agar well and disc diffusion methods (Rad *et al*., 2014).

***In vivo* experiments:** For experimental research, soil was taken from the Department of Botany (University of Karachi) and was sieved using 2mm mesh sieve to eradicate stones or plant debris. Soil contains natural infestation of root pathogenic fungi in which sclerotia of *M. phaseolina* were counted by wet sieving and dilution technique using Sheikh & Ghaffar (1975) procedure. *R. solani* population were estimated by baiting technique using Wilhelm (1955) protocol and *Fusarium* spp., conidial populations were estimated by soil dilution technique in which Nash and Snyder (1962) protocol was used with the slight addition of Andrew & Pitt (1986) method. Soil was filled in a pot (≥1500g) which was placed randomly under natural sunlight in the screen house of the Department of Botany, encapsulated with seed powder of *A. nilotica* which gave best result *In vitro* test. One capsule contains 0.17g of *A. nilotica* seeds powder prepared by capsule filler. Each pot contains fifteen (2.55g seeds powder) and thirty (5.10g seeds powder) capsules, respectively. While, without capsules were taken as control used for comparison and each treatment was replicated thrice. After one day of the amendment of capsules (so it uniformly breakdown in the soil), five seeds of each host such as corn/maize (variety-GOLDEN), peanut/groundnut (variety-Desi #334) and sunflower (variety-Hysun #38) were sown in each pot separately and watered regularly for one month till it fully grown. After one month, plants were uprooted and data of growth parameters were recorded along with the colonization (%) of each root rot fungus was assessed (Narayanasamy, 2011).

Phytochemical analysis

I. Qualitative estimation

Seeds powder of *A. nilotica* (50g) was soaked in 100mL of methanol for 32 hours. The extract was filtered by Whatman No. 1 filter paper for qualitative test of terpenoids, alkaloids, flavonoids, saponin, phenol, tannins, protein, carbohydrate and lipids (Godghate *et al*., 2015; Abbas *et al*., 2016; Siddiqui, 2017).

II. Quantitative estimation

a) Chlorophyll contents and carotenoids analysis: Fresh leaves (0.5g) of peanut, corn and sunflower were homogenized in ethanol (95%) respectively. Afterwards, tested samples were centrifuged at 4000rpm for ten minutes thrice to collect supernatant. Finally, optical density was recorded at 470, 646 and 663nm. Chlorophyll content and carotenoids were calculated by the formula of Lichtenthaler & Wellburn (1983).

b) Total chlorophyll index: Young fully expanded host leaves (corn, peanut and sunflower) were used to measure chlorophyll content index by chlorophyll content meter (model; CL-01 Hansatech, UK).

c) Protein analysis: Leaves (0.1g) of peanut, corn and sunflower were crushed in pre-chilled mortar and pistil containing 5 mL of phosphate buffer. Centrifuge tested samples at 4500rpm for ten minutes; supernatant was treated with 5 mL Bradford assay reagent Bradford (1976). After that optical density was taken at 595nm and protein contents were expressed in µg g^{-1} FW. The standard curve of protein was prepared using Bovin Serum Albumin.

d) Carbohydrate analysis: 100mg fresh leaves were crushed in 5 mL phosphate buffer and centrifuged at 4500rpm. 1 mL of tested supernatant was taken and 2.5 mL anthrone reagent was added and Optical Density was taken at 620nm (Yemm & Willis, 1950). Carbohydrate contents were expressed in µg g^{-1} FW.

e) Lipid analysis: Thimble connected to a soxhlet extractor chamber with flat bottom and pre-weighed about 2.5g of seeds powder of *A. nilotica* were added and then connected with condenser for reflux; petroleum ether (100 mL) was added to the flask and heats it on electric hot plate at 50°C for three hours. After evaporation of petroleum ether, lipid was collected by chilling the flask. Value was calculated by reweighting the flask and lipid content. Lipid percentage (%) was calculated (Anon., 1984).

Statistical analysis

The experimental data was analyzed by using ANOVA (analysis of variance) and LSD (Least Significant Difference) test at P=0.05 and DMRT (Duncan's Multiple Range Test) to compare means (Sokal & Rohlf, 1995).

Experimental results

I. Screening of fungicidal activities of organic medicinal seeds against root rot pathogens by using paper disc and well methods: Medicinal plant seeds recognized as a valuable source of bioactive compounds which linked with strong connection of possessing antifungal activities. Therefore, twenty organic medicinal seeds extracts were used at the concentration of 10,000ppm and 5,000ppm prepared in DMSO, while fungicide (Metiram) at 1000ppm concentration and DMSO taken as control to verify the fungicidal efficacy of seeds extract against *F. solani, F. oxysporum, R. solani* and *M. phaseolina* by using well and paper disc methods.

Results showed that seeds of *P. juliflora* and *M. oleifera* inhibit the mycelial development of *F. solani* and *F. oxysporum* at 10,000ppm but when 5,000ppm concentration was used; it did not show any zone of inhibition in both methods. *W. somnifera* seeds extract at 10,000ppm showed greater controlled mycelial growth of *M. phaseolina* in well method as compared to paper disc method but failed to inhibit the other pathogenic fungi. The interaction between seeds and concentrations were found significant ($p<0.001$). *A. nilotica* seeds extract at 10,000ppm showed excellent suppression of all tested fungi (*F. solani, F. oxysporum, R. solani* and *M. phaseolina*) but no zone of inhibition was recorded under 5,000ppm concentration in both methods. It was astonishing to observe that *D. sisso* and *T. populnea* seeds extract halted the fruiting structure of macro and micro conidia of *F. solani* and *F. oxysporum* at 10,000ppm, only mycelia growth was noticed but no effect was recorded when further concentrations were used. However, in the case of *T. indica* seeds extract, it showed lysis of *F. solani* mycelium. When metiram (fungicide) was used, it inhibits the mycelial growth of *Fusarium* spp. and *R. solani* but no zone of inhibition was recorded in *M. phaseolina* in both methods. While remaining medicinal seeds extracts failed to inhibit the root rot fungi. DMSO (Dimethyl sulfoxide) was taken as control because seed extract was prepared in DMSO but incapable of producing any zone of inhibition against the tested pathogenic fungi were noticed in both methods (Tables 1-4). The interaction between methods and concentrations were found significant ($p<0.001$).

Both methods showed considerable zone of inhibition. However, well method was suggested as better because it has the potential to filled with 100 µL extract in the well which have strong effectiveness to suppress the mycelial growth of pathogenic fungi during the incubation period.

II. Chemical analysis of *A. nilotica* seeds powder by qualitative test: Qualitative chemical analysis of *A. nilotica* seeds powder showed the presence of terpenoids, alkaloids, saponin and carbohydrates in minimum amount as compared to flavonoids, lipid and phenols which were present at greater concentration. Furthermore, the highest concentration of tannin and protein compounds was observed (Table 5).

III. Lipid content of *A. nilotica* seeds powder by quantitative test: The lipid content in the *A. nilotica* seeds powder found to be 33.33%.

Table 1. *In vitro*, growth inhibition of pathogenic fungi by different concentrations (10,000ppm and 5,000ppm) of organic medicinal seeds extract using paper disc and agar well diffusion methods. DMSO served as control and Metiram used as fungicide.

Organic medicinal seeds	Zone of inhibition (millimeter)							
	Fusarium solani							
	Paper disc method				Agar well diffusion method			
	DMSO	10,000ppm	5,000ppm	Metiram	DMSO	10,000ppm	5,000ppm	Metiram
A. nilotica	45.00±0.00	25.33±2.02	45.00±0.00	45.00±0.00	45.00±0.00	21.33±1.85	45.00±0.00	45.00±0.00
A. pavonina	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
A. lebbeck	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
C. fistula	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
C. erectus	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
D. sissoo	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
D. regia	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
F. religiosa	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
L. leucocephala	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
L. usitatissimum	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
M. oleifera	45.00±0.00	39.33±0.66	45.00±0.00	45.00±0.00	45.00±0.00	39.33±0.32	45.00±0.00	45.00±0.00
P. aculeate	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
P. dactylifera	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
P. juliflora	45.00±0.00	39.66±0.32	45.00±0.00	45.00±0.00	45.00±0.00	38.66±0.87	45.00±0.00	45.00±0.00
S. hispanica	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
S. mukorossi	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
T. indica	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
T. populnea	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
T. catappa	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
W. somnifera	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00

$LSD_{0.05}$: Seeds = 0.23; Methods = 0.07; Concentrations = 0.10

Data presented are the mean ± standard error

Table 2. *In vitro*, growth inhibition of pathogenic fungi by different concentrations (10,000ppm and 5,000ppm) of organic medicinal seeds extract using paper disc and agar well diffusion methods. DMSO served as control and Metiram used as fungicide

Organic medicinal seeds	Zone of inhibition (millimeter)							
	Fusarium oxysporum							
	Paper disc method				Agar well diffusion method			
	DMSO	10,000ppm	5,000ppm	Metiram	DMSO	10,000ppm	5,000ppm	Metiram
A. nilotica	45.00±0.00	35.66±1.76	45.00±0.00	39.33±0.57	45.00±0.00	31.00±1.52	45.00±0.00	38.00±0.57
A. pavonina	45.00±0.00	45.00±0.00	45.00±0.00	37.00±1.00	45.00±0.00	45.00±0.00	45.00±0.00	36.33±0.87
A. lebbeck	45.00±0.00	45.00±0.00	45.00±0.00	37.00±0.90	45.00±0.00	45.00±0.00	45.00±0.00	39.00±0.57
C. fistula	45.00±0.00	45.00±0.00	45.00±0.00	37.66±1.66	45.00±0.00	45.00±0.00	45.00±0.00	37.66±0.87
C. erectus	45.00±0.00	45.00±0.00	45.00±0.00	37.33±0.66	45.00±0.00	45.00±0.00	45.00±0.00	38.00±0.57
D. sissoo	45.00±0.00	45.00±0.00	45.00±0.00	37.33±0.32	45.00±0.00	45.00±0.00	45.00±0.00	38.00±2.08
D. regia	45.00±0.00	45.00±0.00	45.00±0.00	38.33±1.00	45.00±0.00	45.00±0.00	45.00±0.00	36.66±1.20
F. religiosa	45.00±0.00	45.00±0.00	45.00±0.00	38.00±1.20	45.00±0.00	45.00±0.00	45.00±0.00	37.66±1.85
L. leucocephala	45.00±0.00	45.00±0.00	45.00±0.00	37.33±0.80	45.00±0.00	45.00±0.00	45.00±0.00	35.00±0.00
L. usitatissimum	45.00±0.00	45.00±0.00	45.00±0.00	37.66±0.32	45.00±0.00	45.00±0.00	45.00±0.00	36.00±1.52
M. oleifera	45.00±0.00	39.00±0.57	45.00±0.00	38.66±1.51	45.00±0.00	38.66±0.32	45.00±0.00	35.00±2.08
P. aculeate	45.00±0.00	45.00±0.00	45.00±0.00	37.00±1.00	45.00±0.00	45.00±0.00	45.00±0.00	38.66±1.45
P. dactylifera	45.00±0.00	45.00±0.00	45.00±0.00	38.00±0.57	45.00±0.00	45.00±0.00	45.00±0.00	36.33±0.87
P. juliflora	45.00±0.00	38.33±0.87	45.00±0.00	38.00±0.57	45.00±0.00	36.00±0.57	45.00±0.00	37.33±1.20
S. hispanica	45.00±0.00	45.00±0.00	45.00±0.00	38.00±0.32	45.00±0.00	45.00±0.00	45.00±0.00	34.33±0.87
S. mukorossi	45.00±0.00	45.00±0.00	45.00±0.00	39.33±0.32	45.00±0.00	45.00±0.00	45.00±0.00	38.00±1.52
T. indica	45.00±0.00	45.00±0.00	45.00±0.00	38.66±0.57	45.00±0.00	45.00±0.00	45.00±0.00	38.66±0.32
T. populnea	45.00±0.00	45.00±0.00	45.00±0.00	38.00±0.32	45.00±0.00	45.00±0.00	45.00±0.00	36.66±1.20
T. catappa	45.00±0.00	45.00±0.00	45.00±0.00	38.33±0.32	45.00±0.00	45.00±0.00	45.00±0.00	37.33±1.20
W. somnifera	45.00±0.00	45.00±0.00	45.00±0.00	39.33±0.30	45.00±0.00	45.00±0.00	45.00±0.00	36.00±1.15

$LSD_{0.05}$: Seeds = 0.55; Methods = 0.17; Concentrations = 0.24

Data presented are the mean ± standard error

Table 3: *In vitro*, growth inhibition of pathogenic fungi by different concentrations (10,000ppm and 5,000ppm) of organic medicinal seeds extract using paper disc and agar well diffusion methods. DMSO served as control and Metiram used as fungicide.

Organic medicinal seeds	Zone of inhibition (millimeter)							
	Rhizoctonia solani							
	Paper disc method				Agar well diffusion method			
	DMSO	10,000ppm	5,000ppm	Metiram	DMSO	10,000ppm	5,000ppm	Metiram
A. nilotica	45.00±0.00	20.33±0.87	45.00±0.00	34.33±1.20	45.00±0.00	15.33±3.21	45.00±0.00	33±1.52
A. pavonina	45.00±0.00	45.00±0.00	45.00±0.00	37.66±0.87	45.00±0.00	45.00±0.00	45.00±0.00	32.66±1.85
A. lebbeck	45.00±0.00	45.00±0.00	45.00±0.00	36.66±0.32	45.00±0.00	45.00±0.00	45.00±0.00	35.00±3.00
C. fistula	45.00±0.00	45.00±0.00	45.00±0.00	36.66±0.66	45.00±0.00	45.00±0.00	45.00±0.00	32.66±1.32
C. erectus	45.00±0.00	45.00±0.00	45.00±0.00	37.33±0.66	45.00±0.00	45.00±0.00	45.00±0.00	35.66±0.32
D. sissoo	45.00±0.00	45.00±0.00	45.00±0.00	35.66±1.32	45.00±0.00	45.00±0.00	45.00±0.00	34.66±0.32
D. regia	45.00±0.00	45.00±0.00	45.00±0.00	37.33±1.20	45.00±0.00	45.00±0.00	45.00±0.00	32.66±2.72
F. religiosa	45.00±0.00	45.00±0.00	45.00±0.00	35.33±1.85	45.00±0.00	45.00±0.00	45.00±0.00	35.00±0.57
L. leucocephala	45.00±0.00	45.00±0.00	45.00±0.00	36.66±1.85	45.00±0.00	45.00±0.00	45.00±0.00	34.66±0.87
L. usitatissimum	45.00±0.00	45.00±0.00	45.00±0.00	37.00±1.15	45.00±0.00	45.00±0.00	45.00±0.00	35.33±0.32
M. oleifera	45.00±0.00	45.00±0.00	45.00±0.00	36.00±0.57	45.00±0.00	45.00±0.00	45.00±0.00	36.00±1.52
P. aculeate	45.00±0.00	45.00±0.00	45.00±0.00	37.00±1.52	45.00±0.00	45.00±0.00	45.00±0.00	35.33±0.66
P. dactylifera	45.00±0.00	45.00±0.00	45.00±0.00	37.00±1.52	45.00±0.00	45.00±0.00	45.00±0.00	35.00±2.31
P. juliflora	45.00±0.00	45.00±0.00	45.00±0.00	36.66±0.32	45.00±0.00	45.00±0.00	45.00±0.00	36.66±0.66
S. hispanica	45.00±0.00	45.00±0.00	45.00±0.00	37.33±0.66	45.00±0.00	45.00±0.00	45.00±0.00	37.00±1.15
S. mukorossi	45.00±0.00	45.00±0.00	45.00±0.00	37.33±0.66	45.00±0.00	45.00±0.00	45.00±0.00	37.00±1.52
T. indica	45.00±0.00	45.00±0.00	45.00±0.00	38.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	36.66±1.20
T. populnea	45.00±0.00	45.00±0.00	45.00±0.00	37.33±1.20	45.00±0.00	45.00±0.00	45.00±0.00	34.33±0.66
T. catappa	45.00±0.00	45.00±0.00	45.00±0.00	37.33±0.66	45.00±0.00	45.00±0.00	45.00±0.00	35.33±0.32
W. somnifera	45.00±0.00	45.00±0.00	45.00±0.00	38.00±1.00	45.00±0.00	45.00±0.00	45.00±0.00	35.66±2.02

$LSD_{0.05}$: Seeds = 0.65; Methods = 0.20; Concentrations = 0.29

Data presented are the mean ± standard error

Table 4: *In vitro*, growth inhibition of pathogenic fungi by different concentrations (10,000ppm and 5,000ppm) of organic medicinal seeds extract using paper disc and agar well diffusion methods. DMSO served as control and Metiram used as fungicide

Organic medicinal seeds	Zone of inhibition (millimeter)							
	Macrophomina phaseolina							
	Paper disc method				Agar well diffusion method			
	DMSO	10,000ppm	5,000ppm	Metiram	DMSO	10,000ppm	5,000ppm	Metiram
A. nilotica	45.00±0.00	22.33±1.76	45.00±0.00	45.00±0.00	45.00±0.00	18.33±0.87	45.00±0.00	45.00±0.00
A. pavonina	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
A. lebbeck	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
C. fistula	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
C. erectus	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
D. sissoo	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
D. regia	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
F. religiosa	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
L. leucocephala	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
L. usitatissimum	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
M. oleifera	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
P. aculeate	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
P. dactylifera	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
P. juliflora	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
S. hispanica	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
S. mukorossi	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
T. indica	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
T. populnea	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
T. catappa	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00	45.00±0.00
W. somnifera	45.00±0.00	38.66±0.87	45.00±0.00	45.00±0.00	45.00±0.00	37.33±1.15	45.00±0.00	45.00±0.00

$LSD_{0.05}$: Seeds = 0.19; Methods = 0.06; Concentrations = 0.08

Data presented are the mean ± standard error

Table 5. Qualitative estimation of *A. nilotica* seeds chemical analysis.

Compounds	Observation	Results
Terpenoids	Reddish-brown coloration appears in the center of test tube	+
Alkaloids	Reddish-brown precipitate appeared in the bottom of test tube	+
Carbohydrate	Dark brown colored with minute amount of precipitate of copper oxide formed in the bottom of test tube	+
Flavonoids	Yellow coloration appeared in the test tube	++
Tannins	Greenish to blackish color appeared in the test tube	+++
Phenols	Solution turns muddy in the test tube	++
Saponin	Foam layer appeared in the test tube	+
Protein	Violet color appeared in the test tube	+++
Lipid	White cloudy emulsion at the top of the water appeared in the test tube	++

Where;
'+' indicates the presence of compounds
'++' shows moderate concentration of compounds
'+++' shows high concentration of compounds

IV) Analysis of soil used for the experimental studies: The soil used for the research was sandy loam containing 77% sand, 11% clay and 12% silt, soil pH was about 7.4≈7.6 along with moisture holding capacity in the soil was 38-42% (Gee & Bauder, 1986). Total nitrogen was 0.09≈1.0% (Guitman *et al.,* 1991) and organic matter present in the soil was 1.2≈1.5% (Sparks, 1996). Natural infestation in the soil contains 8.0≈9.0 sclerotia/g of *M. phaseolina*, 12.0≈14.0% colonization of *R. solani* was recorded and *Fusarium* spp., contain 2400 Colonies Forming Unit (CFU)/g in the soil was estimated.

V) Encapsulation of *A. nilotica* seeds powder in the management of root rot pathogens on the growth improvement of oil yielding crops: Results of thirty capsules of *A. nilotica* seeds powder enhanced the height of shoots of all tested hosts (p<0.001) as compared to untreated (control) plants. Highest shoot and root length as well as fresh and dry weight were observed in peanut plants when thirty capsules mixed in the soil followed by corn and sunflower (p<0.001) plants. *A. nilotica* seeds powder amended with thirty capsules attained better plant growth than fifteen capsules in terms of growth parameters (Fig. 1). Treated plants with *A. nilotica* seeds powder gave noticeable results in the inhibition of root rot fungi when thirty capsules mixed in the soil of peanut and corn plants, it completely suppressed the *M. phaseolina* colonization on peanut and corn plants. *R. solani* mycelium was remarkably controlled in peanut and sunflower plants while in corn minimum inhibition was observed. *F. solani* and *F. oxysporum* colonization were suppressed when fifteen capsules incorporated in soil as compared to control (untreated) condition in which all the pathogenic fungi were recorded in greater

colonization (Fig. 2). Moreover, interaction between capsules and plants were found non-significant. Chlorophyll content was measured in all treated plants which showed better results than non-treated pots. Chlorophyll 'a' was directly proportional to the increasing amount of treatment as numbers of capsules were increased chlorophyll was also observed greater in both concentrations. However, chlorophyll 'b' in peanut and corn plants were recorded higher when amended with fifteen capsules in treated pot but in case of sunflower, highest photosynthetic pigment was observed in thirty capsules treatment followed by fifteen capsules in contrast to control. Total chlorophyll was found highest in fifteen capsules of peanut and corn plants followed by thirty capsules, whereas interaction between capsules and plants were found significant (p<0.05).

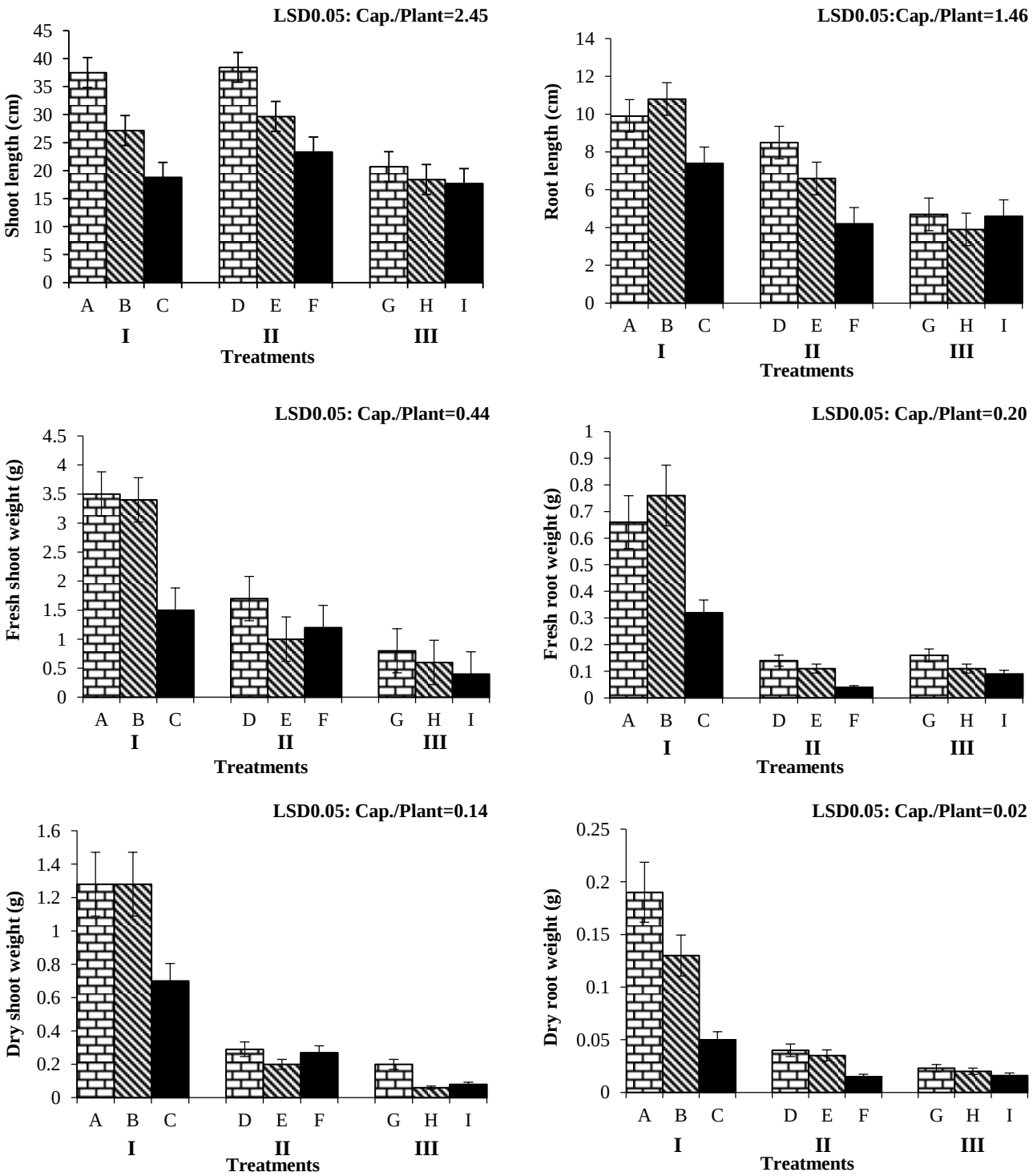

Fig. 1. Effects of encapsulation of *A. nilotica* seeds powder on the growth of oil yielding crops.

Where;

I) Peanut plants; II) Corn plants; III) Sunflower plants: A, D, G = 30 capsules of *A. nilotica* seeds powder. B, E, H = 15 capsules of *A. nilotica* seeds powder. C, F, I = Control (unamended capsules).

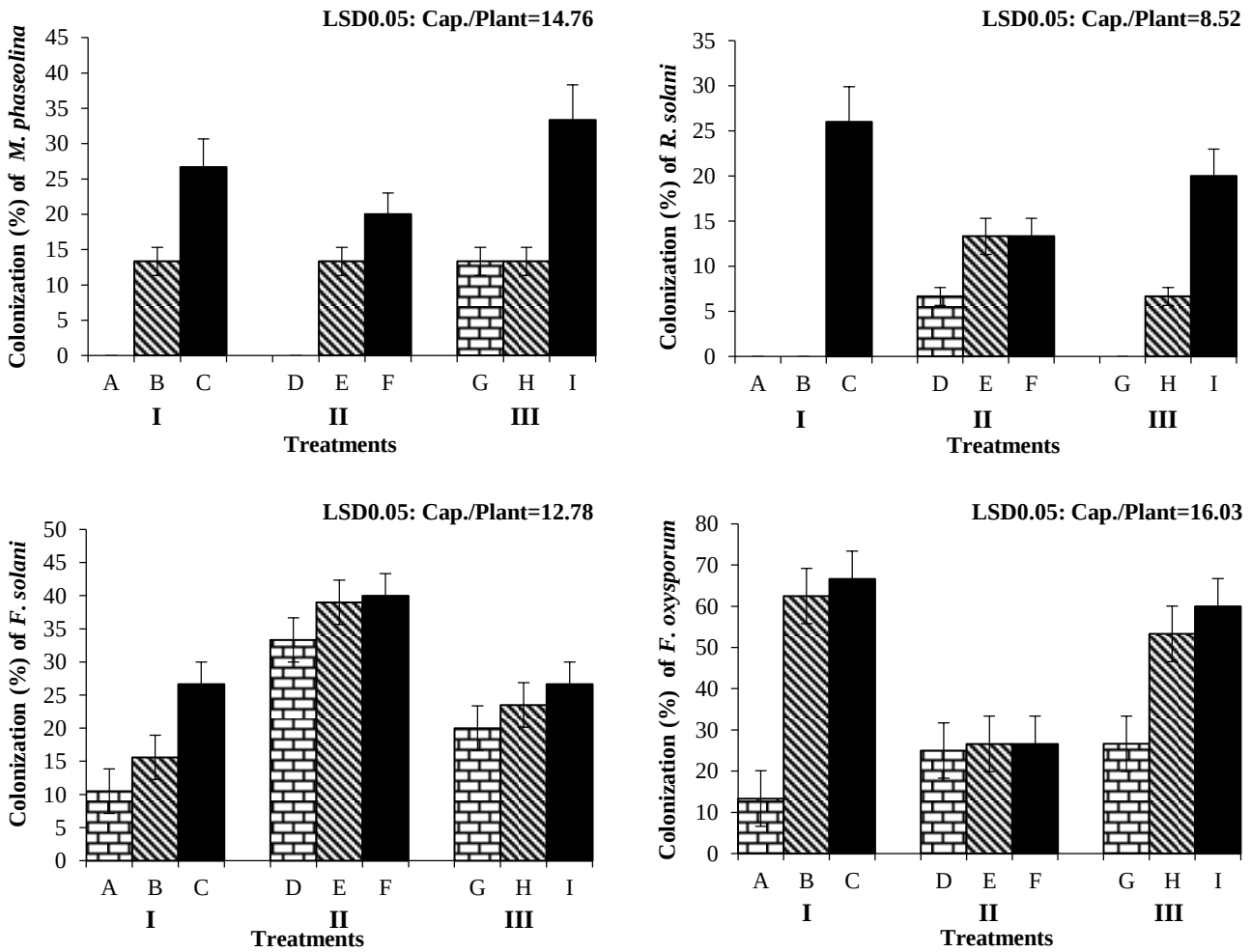

Fig. 2. Effects of encapsulation of *A. nilotica* seeds powder on root pathogenic fungi in the oil yielding crops.

Where;
I) Peanut plants; II) Corn plants; III) Sunflower plants: A, D, G = 30 capsules of *A. nilotica* seeds powder. B, E, H = 15 capsules of *A. nilotica* seeds powder. C, F, I = Control (unamended capsules).

Chlorophyll content index (CCI) was observed by chlorophyll content meter which showed highest at fifteen capsules followed by thirty capsules of corn and sunflower plants, but in case of peanut plant amended with thirty capsules possesses high concentration of CCI followed by fifteen capsules (p<0.001). Using classical or instrument method of recording chlorophyll content similar results were found. Carotenoid content was observed higher in thirty capsules of *A. nilotica* seeds powder of all tested plants (Fig. 3). Carotenoid content showed a direct relationship with the amount of seeds powder amended in the pot, as number of seeds powder capsules were increased, amount of carotenoid was also enhanced. The interaction between them was found to be non-significant. While protein content in sunflower and peanut plants amended with thirty and fifteen capsules gave similar results, but corn plants showed greater concentration of protein when amended with thirty capsules as compared to fifteen capsules. Carbohydrate content in all host plants (peanut, corn and sunflower) observed higher as compared with non-treated plants. Peanut plants treated with thirty and fifteen capsules gave approximately similar results. However, in case of sunflower and corn plants (p<0.05), fifteen capsules were found excellent for the improvement of carbohydrate content followed by thirty capsules as compared to the control plants (Fig. 4). It was striking to note that all treatments of *A. nilotica* capsules (seeds powder) took vigorous part in growth promotion along with the controlled of pathogenic fungi which caused deterioration in roots but amendment of thirty capsules revealed pronounced results in the suppression of root decay pathogenic fungi colonization as well as enhanced the growth of oil yielding crops in contrast to fifteen capsules incorporated in the soil. Although fifteen capsules in each tested hosts (sunflower, corn and peanuts) showed best results in term of growth parameters but relatively less as compared to thirty capsules but striking to observe that it ameliorates the physiological parameters and found highest rather than thirty capsules as compared to control.

Overall result showed the thirty capsules of *A. nilotica* seeds powder amended in soil increased the growth parameter (shoot/ root length and weight; fresh/ dry length and weight) of oil yielding crops but also suppressed the root rot fungal colonization. However, fifteen capsules amended enhanced the carbohydrate, protein and photosynthetic pigments as compared to thirty capsules.

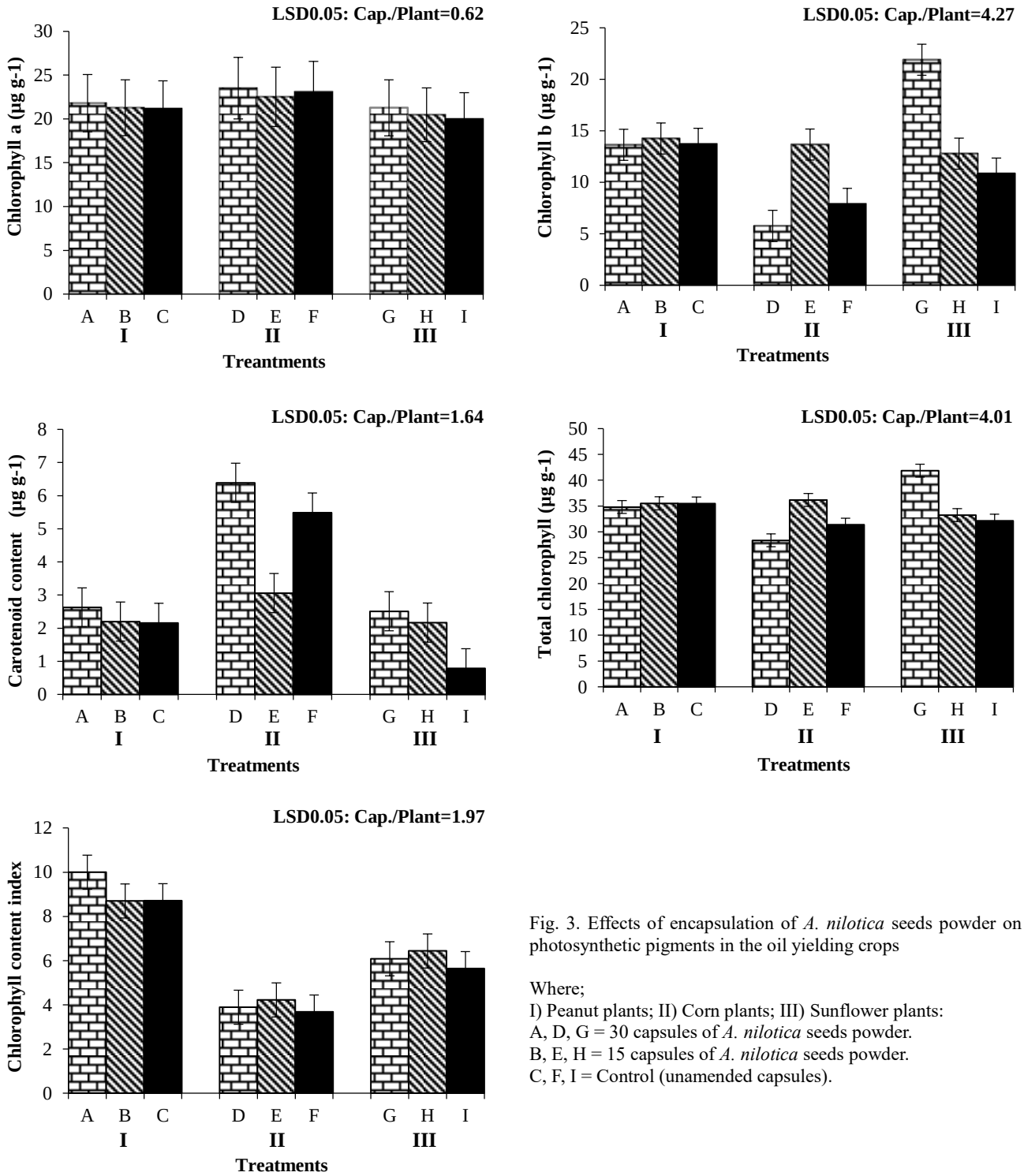

Fig. 3. Effects of encapsulation of *A. nilotica* seeds powder on photosynthetic pigments in the oil yielding crops

Where;
I) Peanut plants; II) Corn plants; III) Sunflower plants:
A, D, G = 30 capsules of *A. nilotica* seeds powder.
B, E, H = 15 capsules of *A. nilotica* seeds powder.
C, F, I = Control (unamended capsules).

Discussion

Plant pathogens are mainly controlled by fungicides as the fast remedy but prolonged used disturb soil ecosystem (Ghazalbash & Abdollahi, 2013). Many researchers worked on the seed extract taking as defensive measures against plant pathogens regarded as eco-friendly method. Ethanol-water *S. aromaticum* seeds extract at 4.0% concentration was found best for the complete growth inhibition against *R. solani* followed by *C. verum*, *P. anisum* and *N. sativa* (Al-Askar and Rashad, 2010) as it contains eugenol compounds contributed the potent antifungal properties by deactivation of enzymes in fungi (Alma *et al*., 2007). Papaya seed extract when used at 200 mg/mL concentration inhibit the mycelial growth of *A. flavus* due to the glycosides and caricin compounds (Eman and El-Zaher, 2014). *M. charantia* L. seeds extract showed antifungal activity towards *F. solani* due to the presence of α-momorcharin (ribosome-inactivating protein) which was analyzed by using optical and fluorescence microscopy which caused disruption in fungal cell wall (Wang *et al*., 2016). Aqueous seeds extract of *M. oleifera* at 75% concentration suppressed *F. oxysporum* but failed to suppress the mycelial growth of *M. phaseolina* and *R. solani* in well and paper disc methods

(Ejaz *et al.,* 2017) due to phenolic compounds like tannins and glycosides possess antimicrobial activities (Kamba & Hassan, 2010). It is reported that presence of tannins prevents the development of microbes (Jamine *et al*., 2007). *A. indica* seed powder at 10.0% concentration remarkably controlled *A. niger*, *A. flavus* and *Rhizopus* species (Hassan *et al*., 2015) due to tetranortriterpenoids compound containing azadirachtin, present in seed kernel (Tabassame *et al*., 2008). Seeds of *L. usitatissimum* ethanolic extract showed significant inhibitory activity against many pathogenic bacteria (Firas, 2007) due to the presence of flavonoids in seeds which disrupt the bacterial membrane (Chaturvedi *et al.,* 2010). Seed extract of *T. indica* showed antifungal activity against *A. niger* and *C. albicans* (El-Siddique *et. al.,* 2006).

Present research of our study showed that methanolic seeds extract of *Acacia nilotica* exhibit as the remarkable result in the suppression of mycelial growth of pathogenic fungi (*Fusarium* spp., *R. solani* and *M. phaseolina*) by using paper disc and well methods. The chemical compounds contain tannin, gallic acid and methyl ester present in the seeds of *A. nilotica* (Ali *et al.,* 2012) were reported to have antifungal, virucidal, antiplasmodial and antibacterial activities (El-Tahir*et al*., 1999 and Hussein *et al.,* 2000). Parekh and his colleague (2006) reported that methanol, ethanol and aqueous usually used solvents for determining the antimicrobial activity in plants. Studies also reported that methanol is a better solvent for more reliable extraction having antimicrobial properties attaining from medicinal plants in contrast to aqueous and hexane extract (Karaman *et al*., 2003). *A. nilotica* contains a variety of inexhaustible bioactive compounds which make this distinctly esteemed plant like alkaloid, glycoside, fatty acid and flavonoid possessing antimicrobial activity (Amjad-ur-Rehman *et al*., 2014). *A. nilotica* mature seed is rich in crude protein/fiber, fat and contain essential mineral elements by using atomic absorption spectrophotometer (Abbasian *et al*., 2015). Seeds analysis in dry form showed that ascorbic acid, phosphorus, thiamine, niacin, iron and protein are present (Chopra *et. al.,* 2013).

Thirty capsules of *A. nilotica* seeds powder amended in soil improved the length and weight of shoot/root of corn, sunflower and peanuts plants but also showed complete suppression of *M. phaseolina* colonization observed in peanut and corn plants, while *R. solani* colonization was completely suppressed recorded in peanut and sunflower plants. However, *Fusarium* spp. was significantly controlled in the roots of all oil yielding crops. Amendment of organic seeds powder approaches as an innovative step to protect roots from the invasion of soil borne pathogens without altering the soil environment (Bonanomi *et al*., 2007; Abdulmoneim & Abu-Zaid, 2011).

Present study showed high amount of chlorophyll content when capsules of *A. nilotica* seeds powder amended in the soil which was also similar result by the amendment of organic products (water hyacinth compost and rice husk biochar) in soil, significantly increased the chlorophyll content of soya bean leaves (Ferdous *et al*., 2018). Total chlorophyll, protein and carbohydrate level increased by 30 and 15 capsules of *A. nilotica* seeds powder in all treated pots as compared to control which was also shown by pineapple leaves and fruit residue amended in soil not only increased the chlorophyll 'a' and 'b' but also soluble sugar and protein contents (Liu *et al*., 2013). Amendment of thirty capsules decreased chlorophyll content but fifteen capsules increase chlorophyll content significantly. High concentration reduced chlorophyll a and b (Gajewska *et al*., 2006). Qualitative study of present results also gave a sign that *A. nilotica* possess antifungal activity by the presence of phenol, flavonoid, tannin and alkaloid compounds which were also reported by Bandaranayake (2002) that presence of tannins, flavonoids and alkaloids present in medicinal plant possess antimicrobial properties. In our present study, encapsulated seeds powder of *A. nilotica* at the rate of 15 and 30 capsules improved the growth of oil yielding crops by controlling plant pathogens. Tariq & Dawar (2013) also used *R. mucronata* parts powder as encapsulation in soil at the rate of 3 to 5 capsules per pot reduced the colonization of pathogenic fungi. Organic amendment provides energy to the soil functions by improving the mineral nutrients, water holding capacity, pH and moisture content which enhanced the growth of crops as well as suppressed the root pathogens (Stone *et al*., 2003).

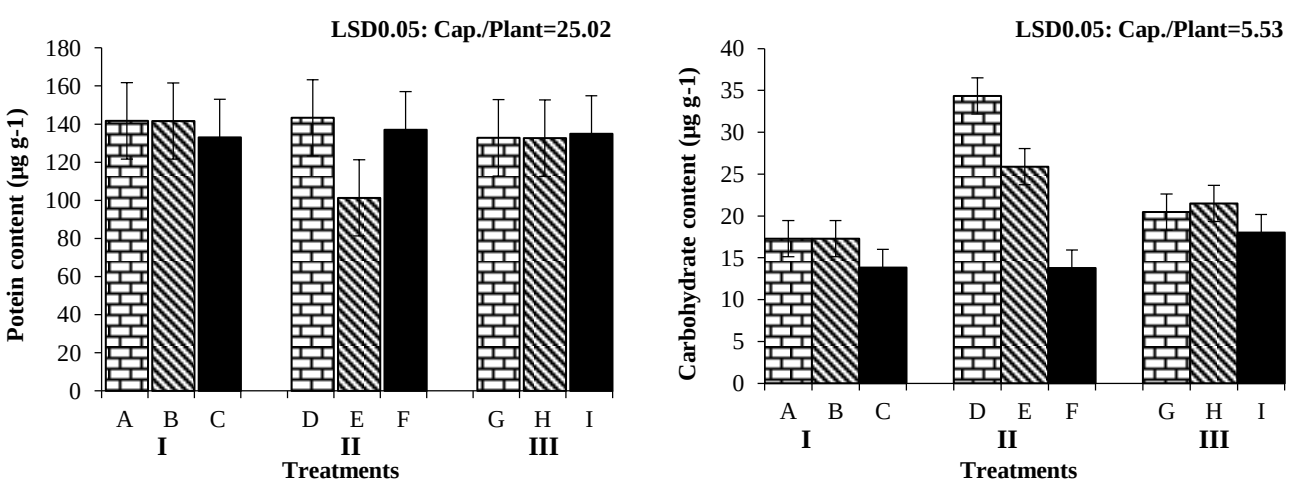

Fig. 4. Effects of encapsulation of *A. nilotica* seeds powder on protein and carbohydrate contents in the oil yielding crops.

Where;

I) Peanut plants; II) Corn plants; III) Sunflower plants: A, D, G = 30 capsules of *A. nilotica* seeds powder. B, E, H = 15 capsules of *A. nilotica* seeds powder. C, F, I = Control (unamended capsules).

Encapsulation is the advancement of organic amendment for the specification of amount of organic matter and plant parts introduced in the soil. Encapsulation not only removed the pathogenic fungi but also improved plant growth by increasing photosynthetic pigments. It is cheap in price as compared to chemical fungicides and easily applicable in commercial agriculture. Therefore, it was strongly recommended for the control of plant pathogens and best growth of plants which was shown by the organic seeds amendment which would be good technique for an ecosystem.

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