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ORIGINAL ARTICLE

Alteration in chemical composition and antioxidant defense potential of essential oil of *Jatropha curcas* L. grown in fly ash-amended soil

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Abstract Fly ash (FA) is a problematic solid waste, possessing essential elements which modifies the properties of soil and improve the plant growth. FA also contains toxic heavy metals, thus there is an urgent need to study the influence of heavy metals present in FA on the growth of plant. The present study was designed to analyze improvement in chemical composition and antioxidant defense mechanism of seed oil extracts from *Jatropha curcas* grown in different doses of FA (0% FA, 25% FA, 50% FA, 75% FA and 100% FA) along with garden soil (GS). A comparison of methyl esters of fatty acids was analyzed in oil extracts using gas chromatography–mass spectrometry (GC–MS). Sixteen compounds were detected, constituting 99.94% of the total oil constituents. Methyl esters of oleic acid, linoleic acid and hexadecanoic acid were the major compounds present. Comparative GC–MS analysis of the three samples showed an increase of 2.15 and 87.84-fold in 25% FA and 50% FA. The antioxidant potential was determined using 2–2′-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing potential (FRAP). Highest scavenging activity and lowest IC_{50} were observed in 50% FA sample followed by 25% FA and 100% GS. Activity of various antioxidant defense enzymes like SOD, CAT, POD and APX was analyzed. Treatment (50% FA with 50% GS) demonstrated enhanced chemical contents and antioxidant defense enzymes. These results suggest the utilization of FA as soil amendment for the growth of non-food crop *J. curcas.* altered chemical composition and antioxidative defense enzymes induced in response to FA amendment to soil reflects its suitability for phytoremediation.

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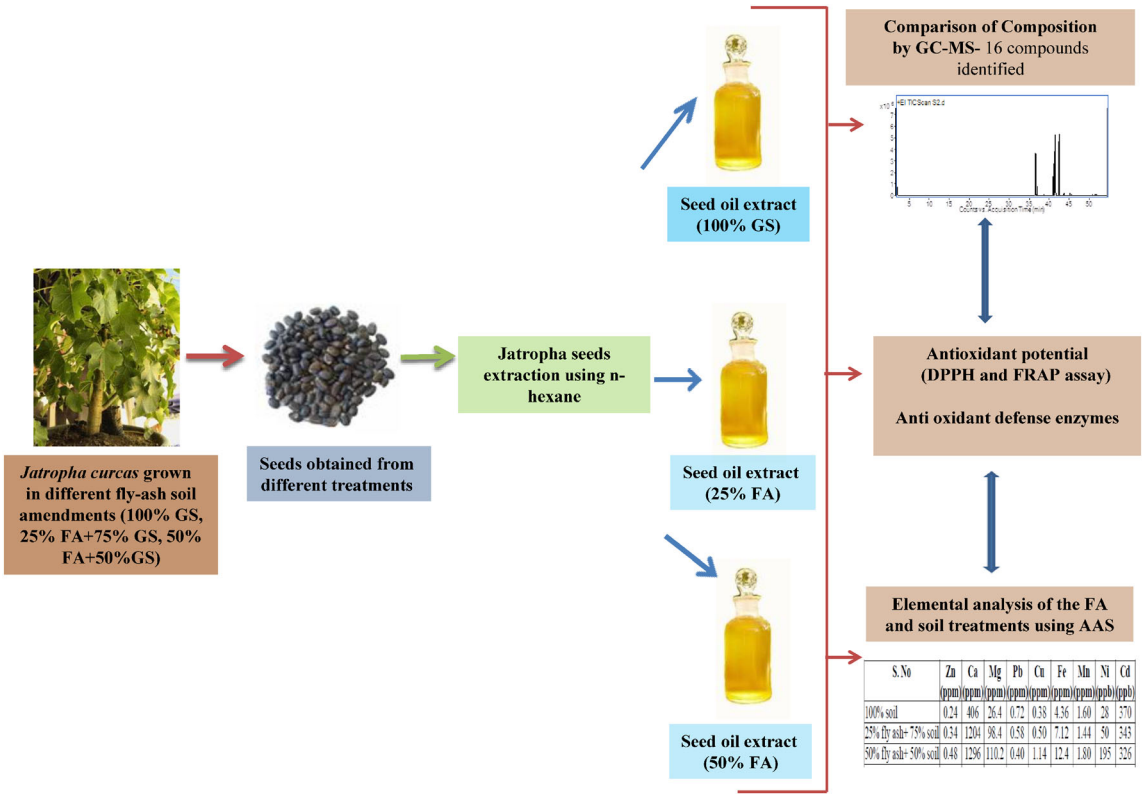
Graphical abstract



Keywords Antioxidant defense mechanism · FAME · Fly ash soil amendments · *Jatropha curcas* · GC–MS

Abbreviations

FA	Fly ash
GS	Garden soil
GC–MS	Gas chromatography–mass spectrometry
DPPH	2–2′-Diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing potential
CAT	Catalase
SOD	Superoxide dismutase
POD	Peroxidase
APX	Ascorbate peroxidase
FAME	Fatty acid methyl ester
ROS	Reactive oxygen species
AAS	Atomic absorption spectrophotometer
DMSO	Dimethyl sulfoxide
EC	Electrical conductance
RT	Retention time
ANOVA	Analysis of variance
SPSS	Statistical product and service solutions

1 Introduction

Fly ash (FA) is the most conversant industrial byproduct produced during coal combustion at high temperatures in thermal power plants (Golewski 2020; Bharath et al. 2017). In India, approximately 169.25 million tonnes of FA is generated, whereas only 107.1 million tonnes is utilized in the year 2016–2017 (Yadav and Fulekar 2018). The major areas of application of FA include its use as a building material, road and dam's construction, manufacturing of cement, used as a soil amendment in agriculture, etc. FA has devastating repercussions on the environment as it degrades arable land, contaminates surface and ground-water and releases its contaminants into the biosphere further aggravates the problem of pollution. Owing to its high disposal costs and management, FA is considered as a problematic solid waste across the globe (Dwivedi and Jain 2014). Besides being regarded as a reprehensible agent to the environment, its sustainable use in combination with soil has been known to deliver striking variations in the growth performance and biochemical properties of plants. Various scientists explore the role of FA amendment in the soil to study the growth and productivity of plants.

Several plants such as *Brassica campestris, Vigna radiate, Cajanus cajan, Helianthus annus, Solenum*

melongata, S. oleracea, Jatopa curcas, etc., are studied at various concentrations of FA (Varshney et al. 2020). FA at a lower concentration enhances the nutrient availability and thus increases the biomass whereas, at a higher concentration of FA used, heavy metal accumulation increases which enhances the antioxidant enzymes present. Physical, biological and chemical properties of the soil are ameliorated with the use of a beneficial quantity of FA in combinations with soil as the FA amended soil serves as a rich source of macro- and micronutrients to plants and has a significant high level of nutrients in the soil such as Ca, Fe, K, Mg and Na which project plant yield and biomass (Mittra et al. 2005; Shakeel et al. 2020). The toxic elements present in FA include Se, Cd, Mo, Ni, Pb and B are a major concern as they may lead to the accumulation of heavy metal in the plant and the associated issues regarding its consumption by human beings (Yunusa et al. 2012). The catastrophic effects of heavy metals present in FA and their influence on plant growth and on the environment cannot be ignored. Consequently, there rises an urgent need for the utilization of FA for plant growth, to study its effect on the composition of plant seed oil and its impact on the antioxidant defense enzymes.

Jatropha curcas (physic nut) is in high demand as it is an oil-bearing hardy plant belonging to the Euphorbiaceae family. The major components of oil include oleic and linoleic acids (Gupta et al. 2012; Senou et al. 2016). Jatropha is native of tropical America and is a genus of 175 plants, shrubs and trees and is now cultivated throughout the globe. The plant has astonishing antimicrobial and antioxidant properties among the medicinal plants (Osoniyi and Onajobi 2003). The seed is rich in oil, which is used as biofuel in many countries. Thus, oil has huge demand in national and international markets. It also contains trypsin inhibitors, phytic acid, saponins and curcin. Previous studies have reported the presence of phytochemicals such as flavonoids, sterols, tannins, terpenes, lectin, hydrocyanic acid, toxalbumine and alkaloids (jatrophine or atherospermidine) in jatropha leaves, stem, latex and root extracts (Omoregie and Folashade 2013). The non-food crop is in high demand as it can be cultivated on polluted soil sites. Heavy metals present in FA possess the capability leading to the production of (reactive oxygen species) ROS (Shahid et al. 2014). Plants as a result of a defense mechanism lead to the production of a high level of secondary metabolites, enhanced antioxidant activity in order to resist oxidative damage. Therefore, the objective of the current study was to assess the chemical composition, total antioxidant activities and antioxidative defense enzyme system from seed oil extracts of *Jatrohpa curcas* grown in different FA amended soil.

2 Materials and methods

2.1 Collection of plant material

The FA used in the present study was collected from the Rajghat power plant, New Delhi, (India). The garden soil (GS) was collected from Amity University, Noida, UP, India. FA was mixed with GS in different concentrations (g/100 mL): 100% GS (Control), 75% GS + 25% FA (25% FA), 50% GS + 50% FA (50% FA), 25% GS + 75% FA (75% FA) and 100% FA in pot culture. The sample size maintained in pot culture was 20 for each treatment and the experiment was repeated three times. Mature seeds (from a one-year-old plant) were collected from each plant sample and identified from Amity Institute of Biotechnology, Amity University-Uttar Pradesh, Noida (India), in August 2016 (Raj et al. 2015). Voucher samples (seeds) were prepared and submitted to the Herbarium for reference purposes. Seeds were collected from all the amendments, thoroughly washed under tap water, and subsequently dried at room temperature. Sample 75% FA and 100% FA were eliminated at this stage as the number of seeds obtained was almost nil.

2.2 Physicochemical analysis of fly ash and garden soil

The air-dried soil, fly ash and fly ash amended soil samples were analyzed for pH, bulk density, electrical conductance and water holding capacity by diluting samples by double distilled water. pH was measured by adding a fixed volume of FA and garden soil in distill water further the readings were noted down using a digital pH meter, electrical conductivity was analyzed using conductivity meter utilizing prepared soil suspension. The bulk density (g/cm^3) was calculated as per Black (1965), and water holding capacity (%) was analyzed as per the method of Pandeya et al. (1968). Moisture content (%) of soil and FA in different treatments was measured by recording the difference in the initial and final weight after drying the sample in an oven at 100 °C for 24 h (Reeb 1999). Total nitrogen, available phosphorus and soil potassium content of garden soil, FA, and different FA and soil amendments were also analyzed (Bisoi et al. 2017).

Elemental composition of GS, FA and FA amended soil samples at different treatments (t-at zero and t-at completion of growth) was done by using atomic absorption spectrophotometer (AAS), Zeenit- 700 from soil testing department, IARI, New Delhi (India). The elements evaluated include Ca, Cd, Cu, Fe, Mg, Mn, Ni, Pb and Zn.

2.3 Extract preparation

Seeds obtained from a one-year-old plant of *Jatropha curcas* grown in different fly ash amendments were used for the preparation of seed oil extract. The seeds obtained from different treatments (100% GS, 25% FA and 50% FA) were separately collected and powdered using porcelain mortar. Crushed seeds (10 g) were filled in the thimble and were extracted in a Soxhlet apparatus at 60 °C for 5 h with 250 mL of solvent n-hexane. The oil extraction proceeded until five cycles of washes to occur where each cycle of extraction includes 60 min. The extracts obtained were concentrated by rotary evaporator and were dissolved in 10 mL/100 mL of Dimethyl sulfoxide (DMSO) which is further maintained at 5 °C in sterile screw-cap vials till further use.

2.4 GC–MS analysis

Jatropha seed oil extracts (100% GS, 25% FA and 50% FA) were converted into methyl esters by adding 0.5 mL of 2 N KOH in methanol and 1.5 mL n-hexane to two drops of the oil sample at 60 °C (Senou et al. 2016). The mixture was vortexed for 2 min and the top layer from the supernatant was transferred into GC vials. The fatty acid methyl esters were quantified by GC–MS. The GC–MS system used was Agilent 7890A series GC system (Agilent Technologies, USA) equipped with a Supelco Omega wax column (30 m*0.25 mm ID, 0.25um thickness, Sigma) and integrated with an Agilent 7000 QQQ MS (Agilent Technologies, USA). To achieve gas chromatography–mass spectroscopic detection and wide separation, an Omega wax column was used. The ionization energy of 70 eV was used and pure helium at 99.99% gas was utilized as a carrier at a flow rate of 1.1 mL/min. The mass transfer line was set at 220 °C and injector temperature was maintained at 250 °C. The oven temperature was programmed initially at 50 °C for 2 min and subsequently 4 °C/min to 220 °C for 10 min with a total 54.5 min run. 1 uL of each sample was injected in the split mode 10:1. Signals produced were recorded in full scan mode (m/z 20–600, 250 scans/milliseconds). The components obtained were subsequently identified by comparing their mass spectra with respect to the authentic samples or the National Institute Standard and Technology (NIST) mass spectral database using AMDIS and mass hunter software (Agilent, USA).

2.5 Determination of the antioxidant activities

2.5.1 DPPH free radical scavenging assay

In-vitro free radical scavenging assay of the *Jatropha curcas* seed oil extracts (100% GS, 25% FA and 50% FA) was quantified by 1,1-diphenyl-2-picrylhydrazyl (DPPH) following the method as suggested by Mensor et al. (2001). The antioxidant potential of each sample was expressed with reference to IC_{50} (concentration of an inhibitor that gives a half-maximal response). The IC_{50} concentration for each sample was estimated from the graph where % inhibition was plotted against extract concentration.

2.5.2 Ferric-reducing antioxidant power (FRAP) assay

The ferric-reducing antioxidant activity of the seed oil extracts was determined as per the method suggested by Benzie and Strain (1999) with certain reconstruction steps. Ascorbic acid was used as an antioxidant standard and positive control. The antioxidant activities of the samples were determined using the ferrous standards. A standard curve of $FeSO_4$ was prepared and the absorbance values were compared. The values obtained were in terms of Ferrous Equivalent (FE), the concentration of extract that provides the same absorbance as 1 mmol L^{-1} ferrous ion.

2.6 Activity of antioxidant enzymes

Superoxide dismutase (SOD), EC 1.15.1.1 activity was analyzed by photochemical method (Choudhury and Choudhury (1985). Catalase (CAT), EC 1.11.1.6 activity was assayed by monitoring the decomposition of H_2O_2 at 240 nm as per the method by Cakmak and Marschner (1992). Peroxidase (POX), EC 1.11.1.7 activity was analyzed by the addition of 100 µL of the crude enzymatic extract to 2.9 mL of the reaction medium, which consisted of 25 mM potassium phosphate buffer (pH 6.8), 20 mM guaiacol and 20 mM H_2O_2 (Kar and Mishra 1976). Ascorbate peroxidase (APX), EC 1.11.1.11 was assayed following the method of Nakano and Asada (1981) by monitoring the rate of ascorbate oxidation at 290 nm. The extracts were used to determine protein content as per Lowry et al. (1951).

2.7 Statistical analysis

The data obtained were subjected to statistical analysis. ANOVA (one-way analysis of variance) and the significance of the difference between means were estimated via Dunnet t test by SPSS 16. Values expressed are means of triplicate determinations ± standard deviation. The IC_{50} value for the DPPH assay was determined using the GraphPad Prism version 8.00 (GraphPad Software, San Diego, USA).

Table 1 Physicochemical parameters of garden soil, fly ash and various fly ash amendments to soil

Parameters	100% GS (Control)	25% FA	50% FA	100% FA
pH	9.2 ± 0.20	8.8 ± 0.05	8.6 ± 0.15	8.0 ± 0.09
EC (Ohm^{-1})	0.10 ± 9.10	0.2 ± 5.42	0.22 ± 3.31	0.3 ± 7.10
Bulk density (g/ml)	1.22 ± 2.11	1.0 ± 1.90	0.98 ± 1.55	0.91 ± 3.37
Water holding capacity (ml/L)	4.1 ± 1.34	4.6 ± 2.25	4.8 ± 2.90	5.6 ± 1.92
Nitrogen (in ppm)	0.19 ± 0.01	0.21 ± 0.02	0.18 ± 0.05	0.09 ± 0.03
Phosphorus (in ppm)	0.09 ± 0.03	0.11 ± 0.05	0.16 ± 0.06	0.14 ± 0.10
Potassium (in ppm)	0.22 ± 0.07	0.27 ± 0.03	0.32 ± 0.03	0.29 ± 0.05

100% GS: Plant grown in 100% garden soil, 25% FA: Plant grown in mixture of 25% fly ash and 75% soil, 50% FA: Plant grown in mixture of 50% fly ash and 50% soil. All results are expressed as mean ± SD from three experiments (n = 3)

3 Results and discussion

In the present study conducted on *Jatropha curcas* grown in FA amended soil, it was observed that 25–50% FA application in soil enhances the morphological and biochemical parameters (pigment content, protein content and sugar content) in the leaves of the plant. Among all FA and soil amendments, 50% FA amendment showed better growth and possess higher amount of chlorophyll-a (0.52 mg/g), chlorophyll-b (0.41 mg/g), carotenoids (0.27 mg/g) and total chlorophyll (0.93 mg/g) content in plant leaves. Uptake of metals, such as Ca, Cd, Cu, Fe, Mg, Mn, Pb and Ni, was analyzed at two different stages, and plant growth and metal uptake were correlated (Raj and Mohan 2018). Results with respect to the growth performance of *Jatropha curcas* such as plant height, leaf area and the number of leaves also revealed that 25–50% FA application in soil showed an improvement in these growth parameters of the Jatropha plant (Raj and Mohan 2016).

3.1 Physicochemical parameters of the garden soil and fly ash

The fly ash and garden soil used for the cultivation of *J. curcas* were found to be alkaline in nature. The bulk density of fly ash was low (0.91 g/mL) as compared to the garden soil (1.22 g.mL) which contrasts with electrical conductance (EC) which was low in garden soil (0.1 Ω^{-1}) and high in fly ash (0.3 Ω^{-1}). High EC in fly ash could be due to the presence of metals such as Fe, Mn, Pb, Cu, Zn, Cr and Si in the FA when compared to the garden soil used. In the present study, the water holding capacity was found to increase when the bulk density decreases. The level of available phosphorus, nitrogen and potassium content was more in fly ash in comparison with garden soil used (Table 1). Fly ash amendment to soil results in significant improvement of electrical conductance, water holding capacity, potassium and phosphorus content which may support plant growth, enhances the yield and thus could be used in agriculture (Sharma and Kalra 2006). The content

Table 2 Elemental composition of garden soil, fly ash and treatments of fly ash in soil at zero time and at completion

Metals	100% GS		25% FA		50% FA		100% FA	
	T = 0	T = F	T = F	T = 0	T = F	T = 0	T = F	T = 0
Zn (in ppm)	0.24	0.22	0.34	0.29	0.48	0.40	1.28	0.66
Ca (in ppm)	406	364	1204	987	1296	1037	1364	786
Mg (in ppm)	26.4	18.5	98.4	49.9	110.2	40.8	127.2	47.2
Pb (in ppm)	0.72	0.627	0.58	0.469	0.40	0.313	0.02	0.014
Cu (in ppm)	0.38	0.35	0.50	0.42	1.14	0.93	1.96	0.69
Fe (in ppm)	4.36	3.44	7.12	4.37	12.4	7.44	20.86	5.14
Mn (in ppm)	1.60	1.33	1.44	0.92	1.80	0.87	3.40	1.34
Ni (in ppb)	28	24.8	50	42.5	195	158.6	400	159
Cd (in ppb)	370	256	343	233	326	181	319	198

100% GS: Plant grown in 100% garden soil, 25% FA: Plant grown in mixture of 25% fly ash and 75% soil, 50% FA: Plant grown in mixture of 50% fly ash and 50% soil. T=0: Elemental composition at Zero−time, T=F: Elemental composition at Completion

Table 3 Chemical composition of seed oil extracts from 100% GS (Control), 25% FA and 50% FA obtained from *J. curcas* grown in different fly ash soil amendments

Sl. No	Compounds	Area			
		RT (min)	100% GS	25% FA	50% FA
1	Tetradecanoic acid, methyl ester	31.45	160,599.48	176,905.02	401,320.57
2	Pentadecanoic acid, methyl ester	33.95	27,862.23	32,373.70	82,808.49
3	Hexadecanoic acid, methyl ester	36.43	21,880,491.85	23,232,475.82	42,398,842.17
4	7-Hexadecenoic acid, methyl ester, (Z)-	36.77	255,124.47	265,501.83	618,966.43
5	9-Hexadecenoic acid, methyl ester, (Z)-	36.93	2,482,265.18	2,559,775.66	5,303,919.16
6	7,10-Hexadecadienoic acid, methyl ester	38.23	90,513.54	93,854.06	218,496.51
7	Heptadecanoic acid, methyl ester	38.64	276,711.97	295,502.91	693,669.80
8	cis-10-Heptadecenoic acid, methyl ester	39.11	154,942.94	165,812.03	381,087.23
9	Octadecanoic acid, methyl ester	40.98	13,534,899.32	14,763,949.74	32,598,941.40
10	Oleic acid, methyl ester/Elaidic acid, methyl ester	41.40	51,248,095.54	53,614,138.89	95,309,806.81
11	Elaidic acid, methyl ester/Oleic acid, methyl ester	41.48	5,094,428.01	5,492,177.86	5,749,033.33
12	Linoleic acid, methyl ester	42.37	45,110,025.90	44,469,843.33	80,163,973.11
13	Linolenic acid, methyl ester	43.57	578,470.75	583,762.24	1,513,212.39
14	Eicosanoic acid, methyl ester	45.03	677,195.87	713,510.72	1,673,298.85
15	Methyl 9-eicosenoate	45.44	236,437.03	262,738.01	574,462.73
16	Oxiraneoctanoic acid, 3-octyl-, methyl ester	50.70	1,056,005.01	1,177,111.78	1,514,525.55

RT Retention time, 100% GS: Plant grown in 100% garden soil, 25% FA: Plant grown in mixture of 25% fly ash and 75% soil, 50% FA: Plant grown in mixture of 50% fly ash and 50% soil

of nitrogen, phosphorus and pH reduced in treatments with fly ash amendment as compared to garden soil used. Pandey et al. (2009) reported that the source of parent coal used determines the FA mineralogical, physical and chemical characteristics. The sulfur content present in the parent coal will lead to varying pH values of FA ranging from 4.5–12. Contrasting results were observed by Kumar and Patra (2012) who reported higher content of nitrogen, phosphorus in garden soil and higher pH values in fly ash samples.

The elemental composition of metals present in garden soil, fly ash and fly ash amended soil treatments at zero time and at the completion of the growth of *Jatropha curcas* using AAS showed an overall increase in the uptake of heavy metal by *Jatropha* plant. It was observed that the level of Pb and Cd decreases as the concentration of fly ash in the soil is increased which may be due to different types of coal used in the power plant. Moreover, even from a single coal-fired power plant, varied compositions of heavy metals can be obtained as suggested by Egemen and Yurteri (1996), whereas the level of other elements, i.e., Zn, Ca, Mg, Cu, Fe, Mn and Ni is in a higher amount in fly ash, and the availability of these ions improves the electrical conductivity of FA. A significant difference was observed in the level of heavy metals present when tested at the time zero and time at completion in 100% GS, 25% FA and 50% FA (Table 2). The percentage absorption of elements by FA (25%) follows the order Mg > Ni > Fe > Mn > Cd > Pb > Ca > Cu > Zn, whereas the order in FA (50%) was Mn > Mg > Cd > Fe > Pb > Ca > Cu > Ni > Zn. There was a significant level of uptake of heavy metal Mg (49.29%), Fe (38.62%) and Mn (36.04) by FA (25%), whereas 51.67% of Mn, 51.38% of Mg and 44.4% of Cd were reported in case of FA (50%). Similar results were reported by Jamil et al. (2009) where heavy metal uptake was increased in roots, stem and leaves by 117, 62 and 86%, respectively, when the garden soil was amended with FA.

3.2 GC–MS analysis of fatty acid methyl esters (FAME)

The GC–MS analysis was performed to analyze the FAME composition of *Jatropha curcas* seed oil extracts. Sixteen compounds were identified for all three samples (100% GS, 25% FA and 50% FA refer to Table 3). The major compounds analyzed in seed oil extracts were oleic acid methyl ester (35.40–36.62% at 41.409 retention time (RT)), followed by linoleic acid methyl ester (29.77–30.37% at 42.377 RT) and hexadecanoic acid methyl ester (15.26–15.87% at 36.433 RT). The major components of oil include oleic and linoleic acids and thus it can be

regarded as unsaturated oil for biodiesel production. Similar observations were reported by Gupta et al. (2012) and Senou et al. (2016). Certain other compounds present in substantial amount were octadecanoic acid methyl ester (9.44–12.10%), elaidic acid methyl ester (3.06–3.69%), 9-hexadecenoic acid methyl ester (1.74–1.97%), oxirane-octanoic acid (0.46–0.73%) and eicosanoic acid methyl ester (0.47–0.62%). Compounds like pentadecanoic acid methyl ester, 7, 10-hexadecadienoic acid methyl ester, methyl 9-eicosenoate, tetradecanoic acid methyl ester, 7-hexadecenoic acid methyl ester were present in trace amounts. Similar results were observed for *J. curcas* seed oil by Senou et al. (2016) and Dabo et al. (2012). There was no significant variation in the level of major component oleic acid methyl esters of the plant, and it was at the maximum level (36.62%) when the plant was grown at 25% FA followed by 100% GS (35.76%) and 35.40% at 50% FA, respectively. The comparative analysis of GC–MS of the three treatments showed multiple-fold change in the amount of component obtained in FA (25%) and FA (50%) sample when 100% GS sample was taken as a reference. A total increase of 2.15-fold has been noted in the sample obtained from 25% FA amendment, whereas the 87.84-fold increase was recorded in the sample from 50% FA amendment in comparison with samples from 100% GS. An increase of 16.1-fold change was observed for pentadecanoic acid methyl ester followed by 11.12- and 10.15-fold changes recorded for methyl 9-eicosenoate when oil extract from 25% FA amendment was analyzed. Oil extract from FA (50%) amendment showed a relative abundance of compounds like pentadecanoic acid methyl ester, linolenic acid methyl ester and heptadecanoic acid methyl ester which possessed a fold change of 197.2, 161.5 and 150.6 which is in contrast to results obtained by Senou et al. (2016). The mechanism resulting in an increase or decrease in the contents of essential oil components of *J. curcas* grown in different treatments is not exactly known, but it could be due to uptake of nutrient and toxic heavy metals present in FA (Kumar and Patra 2012). Moreover, when the plant is under stress conditions, secondary metabolites are produced, and chemical composition may vary. The percentage of compounds present in seed oil also depends on various factors like genotype, stage of maturity, climate type, conditions of cultivation and composition of the soil in different geographical locations (Sanli and Karadogan 2017).

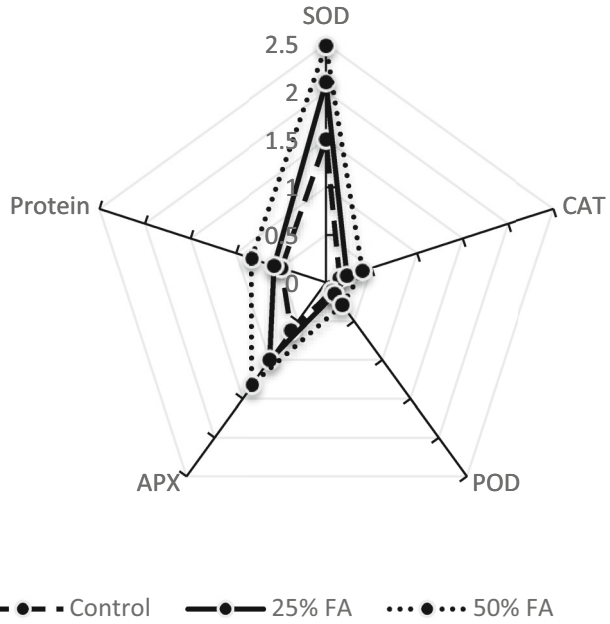


Fig. 1 Spider type visual plot showing quantitative extend of variations in antioxidant enzymes activity and protein content in seed oil extracts of *Jatropa curcas* grown in various treatments of fly ash amended soil (100%GS, 25%FA and 50%FA). SOD: superoxide dismutase, CAT: catalase, POD: peroxidase, APX: ascorbate peroxidase. Data are the mean of three replicates

Table 4 Antioxidant effect of *J. curcas* seed oil extracts grown in different conditions (DPPH, IC_{50})

Concentration (μg ml^{-1})	Average % scavenging			
	100% GS	25% FA	50% FA	Ascorbic acid
10	25.77 ± 0.72	32.47 ± 0.32	37.20 ± 0.41	41.22 ± 0.39
20	40.6 ± 0.83	45.32 ± 0.53	49.85 ± 0.43	56.85 ± 0.46
40	55.4 ± 0.97	54.51 ± 0.77	53.45 ± 0.88	65.24 ± 0.82
60	68.91 ± 1.21	64.39 ± 0.92	70.83 ± 1.15	70.82 ± 1.08
80	74.23 ± 1.72	71.22 ± 1.05	75.02 ± 1.72	89.32 ± 1.91
100	84.67 ± 2.18	86.53 ± 2.30	88.51 ± 2.42	95.35 ± 2.88
IC_{50} (μg ml^{-1})	38.32 ± 0.21	34.48 ± 0.56	28.26 ± 1.25	16.76 ± 0.10

100% GS: Plant grown in 100% garden soil, 25% FA: Plant grown in mixture of 25% fly ash and 75% soil, 50% FA: Plant grown in mixture of 50% fly ash and 50% soil. IC50 values are represented as means ± SD (n= 3)

3.3 Antioxidant assay

Fly ash contains heavy metals which induce oxidative stress in plants and lead to the production of ROS (Arora et al. 2002). Various antioxidant molecules are available in plants which can protect the plants against oxidative damages. Total antioxidant potential of *J. curcas* plant seed oil was analyzed which showed significant variation in different amendments of FA to the soil. In this study, the 50% FA sample exhibited the highest free radical scavenging activity and lowest IC_{50} (28.26 ± 1.25 μg mL^{-1}) followed by 25% FA sample (34.48 ± 0.56 μg mL^{-1}) and 100% GS sample (38.32 ± 0.21 μg mL^{-1}) as shown in Table 4. Contrary to the results obtained, a higher IC_{50} value of 45.60 μg mL^{-1} was reported (Nayak et al. 2016) in *Jatropha curcas* leaf extract. The IC_{50} value of ascorbic acid (reference antioxidant) was found as 16.76 μg mL^{-1}. Ascorbic acid used as a reference antioxidant was found to possess higher vigor. It possesses free radical scavenging of 95.35% at 100 μg mL^{-1} when compared to *Jatropha curcas* oil samples at the same concentration (84.67% in 100% GS, 86.53% in 25% FA and 88.51% in 50% FA). Similarly, higher average % scavenging was reported in *J. pelargoniifolia* essential oil (Aati et al. 2019). The FRAP antioxidant ability of *J. curcas* seed oil extracts from different treatments was evaluated and moderate ferric reduction capability was observed. FRAP values of 177.30 ± 2.66, 191.80 ± 2.14 and 218.67 ± 1.83 mmol L^{-1} ferrous equivalents, respectively, were observed for sample 100% GS, 25% FA and 50% FA, respectively. Similarly, moderate antioxidant activity was reported by various researchers (Oskoueian et al. 2011; Osman et al. 2017). The reducing capabilities of the seed oil extracts toward FRAP were compared to ascorbic acid as the reference standard. Zunjar et al. (2015) reported that the reducing power of the extract due to the presence of certain important compounds present will act as an indicator of its antioxidant potential.

3.4 Antioxidant enzyme activities

To understand the antioxidant defense mechanism operative in *Jatropha curcas*, seed extracts were prepared from plants grown under different treatments of fly ash amended soil and were analyzed for the levels of antioxidant enzymes. The activity of SOD, CAT, POD and APX increased in the 25% FA and 50% FA treatments when compared to the control plant (Fig. 1). Similarly, an increasing trend of protein content was reported from control to 25% FA and further 50% FA suggesting that a higher concentration of FA (50%) is not having a deleterious effect on the level of protein and reflects that heavy metals in fly ash induce the synthesis of stress proteins. Similar results were reported by various researchers in crops such as rice and maize (Bisoi et al. 2017; Arora et al. 2002). However, a reduction in POX and APX enzyme activity during storage of *Jatropha curcas* seeds was reported by Silva et al. (2018). The activity of SOD obtained was higher than that of APX, CAT and POD in the present study. High activity of the SOD enzyme which is the first line of defense increases the content of H_2O_2 in cells, which results in an increase in the activity of antioxidant enzymes which will eliminate the ROS. The H_2O_2 is further broken down to H_2O by POD, and CAT. On the contrary, results were observed in *Spinacea oleracea L* and *Arabidopsis thaliana* where CAT activity decreases and POD, and SOD is enhanced due to the heavy metal stress (Pandey et al. 2009). In plants, a high level of FA stress leads to higher oxidative enzyme activity because of the gradual shift of reductive to oxidative metabolism. The obtained results suggest that heavy metals present in FA are resulting in oxidative stress in seed oil extracts from *Jatropha curcas* plant and increased levels of antioxidant enzymes produced act as a key factor of antioxidant defense mechanism against oxidative injury.

4 Conclusion

The present study concludes that the seed oil extracts from *J. curcas* plant from FA (50%) amended soil improved the physicochemical properties of soil and the biochemical responses when compared to the control plant. A high level of Zn, Ca, Mg, Cu, Fe, Mn and Ni is reported in FA, and the availability of these ions improves the electrical conductivity of FA. The major compounds analyzed in *J. curcas* seed oil extracts include oleic acid followed by linoleic acid and hexadecanoic acid methyl ester. The plant growth in FA and the heavy metal stress may be due to the antioxidant defense mechanism in *J. curcas*. The 50% FA sample exhibited the highest free radical scavenging activity and lowest IC_{50} (28.26 ± 1.25 μg mL^{-1}) followed by 25% FA sample (34.48 ± 0.56 μg mL^{-1}) and 100% GS sample (38.32 ± 0.21 μg mL^{-1}). The seed oil extracts exhibited significant antioxidant defense enzyme activity. SOD activity obtained was higher than that of APX, CAT and POD activity in the present study. The results obtained suggest that FA-containing heavy metals lead to oxidative stress in seed oil extracts and an enhanced level of antioxidant enzymes produced. However, further insight is needed to understand the relationship between heavy metal stress and antioxidant behavior. The present study demonstrated altered chemical composition, total antioxidant activities and antioxidative defense enzyme system in seed oil extract of *J. curcas* induced in response to FA

(50%) amendment to soil, thus suitable for phytoremediation.

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Declarations

Conflict of interest The authors declare that they have no potential conflict of interest in the publication.

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