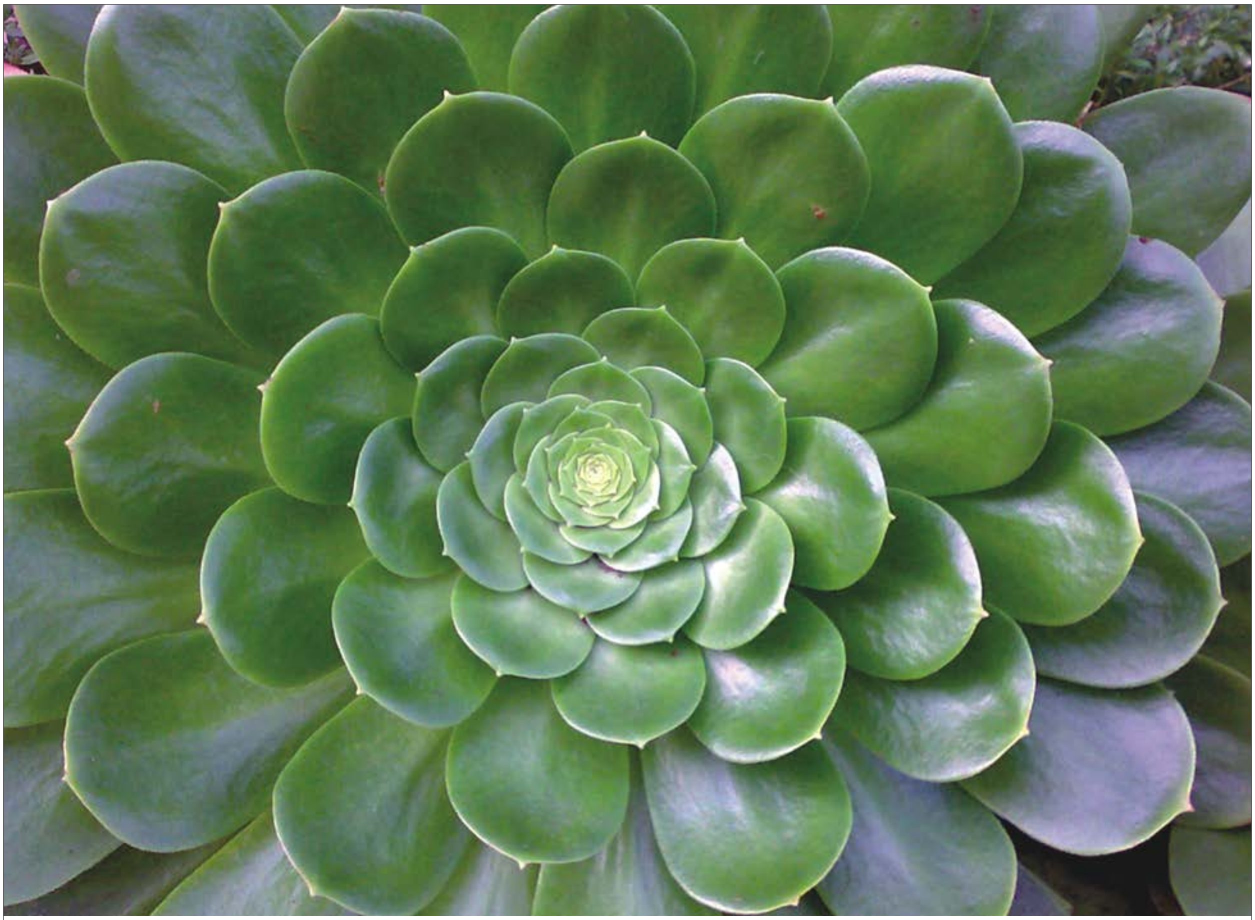

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Research Article
Soybean Plants Treated with Vesicular Arbuscular Mycorrhiza Fungi Exhibit Enhanced Plant Growth and Nutraceutically Important Metabolites

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Abstract

Background and Objectives: Soybean (*Glycine max*. (L.) Merr) is a highly valued source of protein and vegetable oil, grown extensively in India as well as globally and gaining importance as a nutraceutical. The objective was to study the influence of vesicular arbuscular mycorrhiza (VAM) on growth, phenolic compound, isoflavone folic acid content and antioxidant activities to enhance nutraceutical value of soybean. **Materials and Methods:** Soybean seeds, variety JS-335 were germinated in association with VAM fungi *Glomus fasciculatum* (*G. fasciculatum*) and *Glomus mosseae* (*G. mosseae*) grown in pots containing sand-soil mixture (1:1) with respective VAM inoculants and controls. Plants were grown for 9 weeks with weekly observations and data were recorded with respective height of each plant, corresponding dry weight, pods weight per plants. The control and treated plants were analyzed for phenolic compound, flavonoids and antioxidant activities. And isoflavone content was quantified by using HPLC method. **Results:** Treatment of VAM resulted in increase in fresh weight (approx. 2.5 fold) and dry weight of 0.8-2.2 folds. Similar increase in seed weight per plant was obtained under VAM treatment. Flavonoid levels, folic acid, isoflavone content and phenolic contents were higher in the VAM treatments in comparison to control. Soybean extract showed great ability to scavenge free radicals and antioxidant activities. **Conclusion:** Seeds of soybean showed the presence of bioactive compound such as flavonoid, folic acid, phenolic, isoflavone and antioxidants. Profiling of phytochemicals in soybean seeds in response to VAM treatment will help to select suitable cultivars for their utilization in the preparation of value added processed soy products, thereby enhancing the health benefits of soy and soy-based foods for human consumption.

Key words: *Glycine max*, folic acid, isoflavones, nutraceutically, vesicular arbuscular mycorrhiza

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybean (*Glycine max*. (L.) Merr) is a highly valued source of protein and vegetable oil and grown extensively in India and other parts of the world. World production of soybean in 2015 was 317.0 million metric tons while the world production is expected to increase to 324.2 million metric tons by 2020[1]. In order to enhance the productivity of soybean to meet the growing demands, it is necessary to obtain soybean varieties resist to several agronomic stresses and also from the point of view of protection from pests, diseases and weeds[2].

Beneficial microbes namely Vesicular-Arbuscular Mycorrhizal (VAM) exhibit symbiotic association with plants for mobilizing the nutrients and water for increased yield by enhanced growth and productivity[3,4]. Plant growth stimulation induced by VAM fungi influences host uptake of soil phosphorus (P) primarily, but also of other minerals[5].

Folic acid (Vitamin B9) is an important water soluble vitamin. It is used to treat conditions associated with folic acid deficiency, ulcerative colitis, liver diseases, alcoholism and kidney dialysis[6]. Therefore, enhanced level of folic acid in soybean will be valuable for enhancing the nutraceutical value. Phenolic compounds are plant derived antioxidants, soybean and soybean products contain phenolic compounds of which 72% is isoflavones[7]. Phenolic compounds possess metal chelating and radical scavenging properties[8]. Antioxidants, free-radical scavengers play significant pathological role in human disease[9]. It has been observed that the intake of antioxidants containing soy foods was associated with a reduced cardiovascular risk resulting from lower blood pressure and homocysteine[10]. Total phenolic content is a potential candidate as a selection criterion for antioxidant activity in soyabeans[11]. Soybean produces most abundant flavonoid, rutin also known as vitamin P[12]. Rutin is a scavenger of hydroxyl as well as peroxyl radicals and its biological activities have been widely explored including antibacterial, anti-inflammatory, anti-allergic, antiviral, anti-protozoal and anti-tumor properties[13,14]. Soya isoflavones (Genistein and daidzein) has estrogenic activity in mammals and is reported to prevent cardiovascular disease, prostate and breast cancers, osteoporosis and relieves menopausal symptoms[15]. Therefore, enhancement of useful phytochemicals of soybean will add nutraceutical value to crop.

During the present study, inoculums of two species VAM fungi, *Glomus fasciculatum* and *Glomus mosseae* were separately applied to the rhizosphere of the genotypes of soybean to investigate their beneficial effects on the growth and productivity of the plants in greenhouse conditions. Enhancement of nutraceutical value was determined by measuring the total phenolic content and concentration of flavonoids in soybean as well as examining the rutin content, antioxidant activity and folic acid content of soybean seeds in VAM treated plants.

MATERIALS AND METHODS

The green house study was conducted at the Dayananda Sagar Institution, Bengaluru Campus, from September-December, 2015 as field experiment. This current study emphasizes on the effect of VAM fungi on physiological and agronomical characteristics of soybean plant. In addition, the determine the level of phenolic, antioxidant, folic acid and isoflavones in VAM treated soy seeds and experimental study was carried out at the laboratory in department of biotechnology.

Materials: DPPH, gallic acid, quercetin, ABTS were purchased from Sigma-Aldrich (MO, USA). All the chemicals were used of analytical grade and highest purity. The other chemicals were from Himedia and SD Fine Chemicals, Mumbai, India.

Preparation of standard solutions: The standard stock solution (1000 $\mu g\ mL^{-1}$) of quercetin and gallic acid (100 $\mu g\ mL^{-1}$) were prepared by dilution in absolute alcohol and water, respectively. The dilute standard solutions of concentration,100-1000 $\mu g\ mL^{-1}$ of quercetin and 20-100 $\mu g\ mL^{-1}$ of gallic acid, were prepared from above stock solution.

Plant material: Soybean genotype JS 335 was obtained from Tamil Nadu Agriculture University (TNAU) Coimbatore. It is high yielding genotype and cultivated mainly in the Central as well as in the Northern parts of India. Explant was surface disinfected with 70% ethanol for 2 min, followed by 10 min in 5% sodium hypochlorite (v/v) treatment.

VAM culture: The inoculums of *Glomus fasciculatum* and *Glomus mosseae* were obtained from Gandhi Krishi Vigyana Kendra, University of Agricultural Sciences and Bangalore, India.

Multiplication of VAM: Firstly, *Eleusine coracana* seeds were sown in pot with soil along with the inoculums of

G. fasciculatum (GF) and *G. mosseae* (GM) which were layered 2 cm below the top soil prior to sowing of soybean seeds under greenhouse condition. After 2 weeks the plants were pulled up and the multiplied VAM fungi has been used for further studies of pot experiments.

Greenhouse trials: Soybean JS-335 was sown in pot with soil. The inoculums of *G. fasciculatum* and *G. Mosseae* were, multiplied or grown separately. The VAM inoculum was layered 2 cm below the top soil surface at the time of transfer of cotyledons as explants. Plants were irrigated regularly to maintain the desired moisture in soil under greenhouse condition. Observations were made regarding plant height, number of branches, leaves, length of leaf, width of leaf, number of pods, germination percentage, number of seed per pods the dry and fresh weight of shoots and roots were recorded in 60 days old plants.

Solvent extraction: The soy seeds samples were extracted as described by Sakthivelu *et al.*[16]. Sample was then filtered and transferred in fresh vials. The extract was used for flavonoid, phenolic, folic acid, rutin, isoflavone and antioxidant assays.

Determination of total flavonoid content: Total flavonoid content was measured with the aluminium chloride colorimetric assay[17]. The calibration curve was plotted using standard quercetin.

Determination of total phenol content: The total phenolic content was determined as described by Velioglu *et al.*[18] with minor modifications. Total phenolic content was expressed as gallic acid equivalents (mg GAE/100 g of soybean) through standard calibration curve of freshly prepared gallic acid.

Determination of folic acid: The assay was performed by simple spectrophotometric method[19]. The absorption of complexation was measured at 460 nm using UV-Visible spectrophotometer.

Determination of rutin

HPLC Analysis: The HPLC analysis was conducted following the method of Walsh *et al.*[20]. Separation of isoflavones was achieved by Bondapak C_{18} reversed phase HPLC column (250×4.6 mm and 5 µm internal diameter) and 20 µL samples were injected using a Rheodyne 7125 injector, solvent flow rate was maintained at 1 mL min^{-1} the wave length of UV detector was set at 235 nm. Rutin from SD fine chemicals was used as reference standard.

Determination of isoflavones by FTIR: About 5 mg of powdered material was grounded with 300 mg of potassium bromide and then pressed to form a translucent pellet. The infrared spectrum of the material was recorded using Shimdazu (Cary-630) Fourier transform infrared spectrophotometer in the range of 400-4000 cm^{-1} compared the IR spectrum with similarly recorded spectrum of the standards (Daidzein and Genistein).

Isoflavone analysis

HPLC Analysis: The HPLC analysis of isoflavone was carried out by the method of Walsh *et al.*[20]. Isoflavone identification was done using authentic standard of Daidzein and Genistein.

Evaluation of antioxidant activity

DPPH free radical scavenging activity: The DPPH-free radical scavenging capacity of legume extracts was evaluated according to the method of Chen and Ho[21] with slight modifications. The percentage of DPPH radical was determined according to the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity was performed as described by Halliwell[22]. The percentage of inhibition of hydroxyl radicals is calculated by the formula mentioned above for DPPH method.

Nitric oxide scavenging activity: Nitric oxide scavenging assay was carried out as per the method of Sreejayan and Rao[23]. The percentage of inhibition of nitric oxide scavenging was calculated by formula mentioned above for DPPH method

ABTS assay: The assay was performed according to the method of Auddy *et al.*[24]. Percentage of inhibition of ABTS was calculated by formula mentioned above for DPPH method.

Iron to iron reducing activity: The assay was performed according to the method of Benzie and Strain[25]. Percentage of inhibition was calculated by formula mentioned above for DPPH method.

Statistical analysis: Results obtained were mean of three determinants. Analysis of variance was carried out on all data at $p<0.005$ using one-way ANOVA.

RESULT AND DISCUSSION

Influence of VAM on the growth of soybean: In this study JS 335 soybean genotypes were used because it occupies more than 50% of the growing areas in India. JS 335 has resistance against girdle beetle and stem fly and tolerance to moisture stress conditions. The present study revealed differences in plant performance between the physiological and agronomical characteristics of variety of soybean JSS-335 under the greenhouse cultivation. Inoculation of both the VAM fungi separately to soybean variety (JS-335) resulted in significant increase in plant height and shoot length of soybean plants (Fig. 1 and 2). Treatment of VAM (GF and GM) resulted in increasing fresh weight (approximately 2.5 fold) and dry weight of 0.8-2.2 folds under VAM treatments. Similar increase in seed weight per plant was obtained under VAM treatment (Table 1). The effect of *G. fasciculatum*, inoculation was found to be superior to *G. mosseae* in the variety of soybean tested. Throughout the experiments, VAM fungi had a beneficial effect on plant growth. Similarly, Kyriazopoulos *et al.*[26] reported the effects of Arbuscular Mycorrhizal Fungi (AMF) on growth characteristics of *Dactylis glomerata* wherein they have observed significantly higher shoot dry weight, tiller height and number of leaves in comparison to the un inoculated plants. Many studies on AMF inoculation have also shown positive effects on various plants, but there are differences in response of the varieties, cultivars or species to various AMF species[27-29].

Influence of VAM on total flavonoid content (TFC): The total flavonoid content was highest (0.463 mg quercetin/g) in soybean seeds in *G. fasiculatum* treatment compared to untreated control soybean genotypes (Table 2). Surprisingly lower level of flavonoid was found in *G. Mosseae* treated soy seeds. Similarly, flavonoids from different plant sources have shown free radical scavenging activity and protection against oxidative stress[30]. Flavonoids are present in routine diet in the form of fruits and vegetables. Josipovic *et al.*[31] reported the total flavonoid content (TFC) of soybean genotypes varied from 0.433-0.658 mg g^{-1} of dry weight, expressed as catechin equivalent. Fidrianny *et al.*[32] reported that the total flavonoid of ethanolic extract from four species legumes (soybean, red kidney bean, Bogor peanut and peanut) were 1.64, 2.26, 1.65 and 6.42 mg QE/100 g, respectively. Thus significant enhancement in flavonoid in the present study adds value to the soybean crop from nutraceutical point of view.

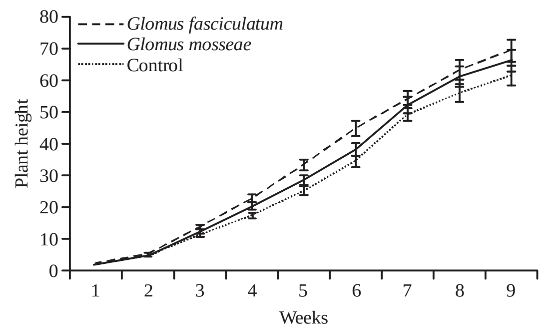

Fig. 1: Soybean plant (JS-335 variety) treated with *G. fasciculatum* and *G. mosseae* and its effect on plant height

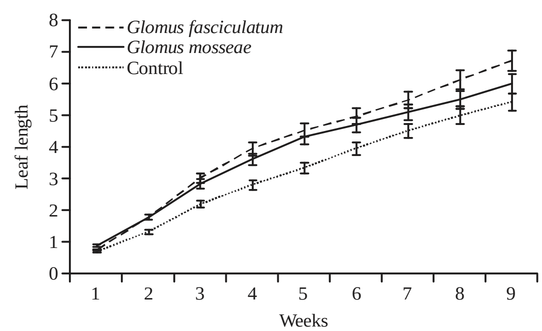

Fig. 2: Soybean plant (JS-335 variety) treated with *G. fasciculatum* and *G. mosseae* and its effect on plant shoots

Table 1: Yield performance of the soybean genotypes treated with the VAM fungi (GF and GM)

Treatments	Number of pods/plant	Fresh weight of pods/ plant (g)	Dry weight of pods/ plants (g)	Fresh weight of each plant (g)	Dry weight of each plant (g)	Weight of seeds per plant (g)
JS-335 (control)* Untreated	5±0.78	2.27±0.67	1.38±0.34	3.91±0.74	1.13±0.13	0.70±0.07
JS-335 treated with (*G. fasciculatum*)-GF	14±1.03	6.56±0.87	3.59±0.54	10.15±0.94	2.60±0.28	2.16±0.24
JS-335 treated with (*G. mosseae*)-GM	9±0.94	3.83±0.55	2.24±0.67	6.63±0.89	1.95±0.27	1.26±0.17

*control: Without treatment of VAM ,±standard deviation

Table 2: Flavonoid contents of soybean seed obtained from VAM treatment

Soybean variety (JS-335) treated with VAM	Flavonoid content (mg of quercetin/g)
*Control	0.418±0.005
G. fasciculatum	0.463±0.009
G. mosseae	0.379±0.017

*Control: Without treatment of VAM,±standard deviation, $p<0.005$

Table 3: Total phenolic contents of soybean seed obtained upon VAM treatment

Soybean variety (JS-335) treated with VAM	TPC (mg of GAE/g)
*Control	1.818±0.078
G. fasciculatum	2.712±0.012
G. mosseae	1.918±0.047

*Control: Without treatment of VAM,±standard deviation, $p<0.005$

Table 4: Hydroxyl reducing activity of soybean seeds in plants treated with VAM. Hydroxyl reducing activity was represented as percent inhibition

Concentration (µg)	Inhibition* (%)		
	Control**	GF	GM
100	35.93±2.12	86.15±0.95	41.78±3.69
200	41.40±1.51	88.15±1.99	60.25±1.15
300	58.95±1.52	90.05±3.55	63.05±1.43
400	63.40±0.52	91.07±3.10	67.05±0.91
500	67.17±1.50	92.00±2.00	68.64±2.19

**Control: Without treatment of VAM,±standard deviation, GF: *G. fasciculatum*, GM: *G. mosseae*, $p<0.005$

Influence of VAM on total phenolic content: Xu and Chang[33] reported the range of total phenolic content of 2.07-2.90 mg GAE/g in 28 soybean samples. The total phenolic content of methanol extracts of soy seed control and VAM treated of plants was presented in Table 3. The value was expressed in terms of GAE using the standard curve equation. The total phenolic content was highest in VAM treated soybean genotypes 2.712 mg GAE/g compared to, 1.818 mg in control. In this study the VAM treatment had beneficial effect in enhancing phenolic content in soybean seeds. According to Mujic *et al.*[34], total phenolic content ranged from 0.87-2.16 mg GAE/g. Higher values for phenols were found in black and brown coloured soybeans[35]. The content of phenolics present in the VAM treated soybean is in the range reported by the earlier studies[33,34].

Antioxidant assays

Hydroxyl radical scavenging activity: Hydrogen peroxide (H_2O_2) is generated *in vivo* by several oxidative enzymes and by dismutation of superoxide radical catalyzed by superoxide dismutase. Hydrogen can be toxic to cell because it may give rise to hydroxyl radical in the cells[22]. H_2O_2 scavenging activity of the extracts was shown in Table 4. Enhanced hydroxyl radical scavenging activity by VAM treatment was evident, which is a desirable attribute towards nutritional enhancement of soybean seeds obtained by VAM treated plants.

Table 5: Nitric oxide scavenging activity of soy seed in VAM treated plants Nitric oxide activity was represented as percent inhibition

Concentration (µg)	Inhibition* (%)		
	Control**	GF	GM
100	48.64±1.60	20.64±1.03	78.41±1.94
200	53.12±1.51	25.80±0.91	78.94±1.08
300	60.74±1.67	43.22±1.39	79.67±2.12
400	62.93±2.10	45.16±2.00	80.48±0.94
500	70.16±1.88	51.61±0.89	82.64±1.50

**Control: Without treatment of VAM,±standard deviation, GF: *G. fasciculatum*, GM: *G. mosseae*, $p<0.005$

Table 6: ABTS scavenging activity of soybean seeds from different VAM treated plants. *ABTS activity was represented as percent of inhibition

Concentration (µg)	Inhibition* (%)		
	Control**	GF	GM
100	11.49±0.97	24.99±1.96	19.63±0.97
200	19.72±1.90	45.96±1.17	21.64±0.55
300	29.43±1.55	64.87±1.60	32.14±1.56
400	37.82±0.99	77.19±1.48	40.19±2.08
500	45.71±0.86	92.14±0.98	50.14±1.51

**Control: Without treatment of VAM,±standard deviation, GF: *G. fasciculatum*, GM: *G. mosseae*, $p<0.005$

Nitric oxide scavenging activity: Reactive nitrogen species (RNS) are responsible for the oxidative stress and have been implicated in the pathogenesis of several diseases, including cancer, diabetes and renal disease. Accordingly, the scavenging of NO radical may be a promising indicator in screening healthy foods[36]. NO scavenging ability of extract of control and treated was presented in Table 5. Seed extract of soybean plant treated with *G. mossease* (JS-335) exhibited maximum scavenging ability than control. Thus enhancing the nutraceutical potential of soya seeds obtained upon VAM treatment of plants.

ABTS assay: The ABTS assay was employed to measure the antioxidant activity of the soybean seed extract because it provides a good estimate of the antioxidant activity of pure compounds and complex matrices[37]. The soya seeds extract displayed antioxidant activities as it was able to scavenge the ABTS radicalcation. The soybean seeds extract had a scavenging activity on ABTS radicals in a dose dependent manner from 100-500 µg. Percentage of inhibitions of treated extracts of *G. mosseae* (JS-335) and *G. fasciculatum* (JS-335) has highest compared to control (Table 6). Thereby, showing potent antioxidant effect in seeds of VAM treated soybean

Table 7: DPPH Radical scavenging activity of soybean seeds in VAM treated plants. *DPPH Radical scavenging activity was represented as percent of inhibition

Concentration (µg)	Inhibition* (%)		
	Control**	GF	GM
100	13.42±1.47	19.75±2.06	10.90±0.66
200	15.42±0.95	27.10±1.60	21.00±1.00
300	16.62±1.51	32.71±0.98	25.20±1.47
400	18.71±2.08	40.12±1.02	26.71±0.99
500	19.81±1.03	42.18±1.51	28.00±1.00

**Control: Without treatment of VAM, ±standard deviation, GF: *G. fasciculatum*, GM: *G. mosseae*, p<0.005

Table 8: Ferric iron reducing activity of soybean seeds in VAM treated plants

Soybean variety (JS-335) treated with VAM	Inhibition of ferric iron (%)
*Control	26.48±0.816
G. fasciculatum	31.48±1.584
G. mosseae	27.77±1.563

*Control: Without treatment of VAM, ±standard deviation, p<0.005

Table 9: Folic acid content in seed of soybean (*Glycine max*) upon VAM treatment

Soybean variety (JS-335) treated with VAM	Folic acid (µg mg^{-1}) DW
*Control	40±0.816
G. fasciculatum	74±1.000
G. mosseae	90±1.000

*Control: Without treatment of VAM, ±standard deviation, p<0.005

Table 10: Rutin content in seeds of soybean (*Glycine max*) under VAM treatments

Soybean variety (JS-335) treated with VAM	Rutin present in seeds (%)
*Control	0.24±0.015
G. fasciculatum	0.41±0.010
G. mosseae	0.81±0.035

*Control: Without treatment of VAM, ±standard deviation, p<0.005

plants. Improved ABTS activity by VAM treatment was obvious, which is a value addition towards nutritional enhancement in soya seeds.

Inhibition of DPPH radical: The DPPH is a stable lipophilic free radical that is used to determine the proton-scavenging activity of the various soybean extracts. The DPPH radical scavenging activity of methanol soybean crude extract revealed antioxidant potency based on percentage of inhibition. The lower percentage inhibition value indicates stronger antioxidant activity in the sample (Table 7). In this study the antioxidant property demonstrated by inhibitions of DPPH radical is attributed to phenolic and also flavonoids percentage in the seeds.

Ferric ion reducing power of antioxidant: In this context, antioxidant activity of VAM treated soybean was significantly higher compared to non-treated soybean plant (Table 8). Percentage of inhibition of ferric iron was highest in *G. fasciculatum* treated soybean plant extract followed by *G. Mosseae* and controls. Similar to DPPH, ABTS assay the ferric ion reducing power was also enhanced by VAM treatment of GF when compared to untreated control and GM treatment.

Prvulovic *et al.*[38] studied antioxidant and phenolic content of soybean seed extracts and they have found highest antioxidant activity in acetone extract. Samruan *et al.*[39] studied the antioxidant activity of soybean and fermented soybean extract. From both ABTS and DPPH assay, the antioxidant activity of soybean and fermented soybean water crude extract showed higher antioxidant activity than ethanol extract, respectively. For the first time authors showed enhancement of antioxidant property in VAM treatments in soybean which can be integrated in to agronomic management practise to enhance the crop yield and value.

Influence of VAM on folic acid determination: Folic acid concentration was found to be 40, 74 and 90 µg mg^{-1} dry weight (Table 9). Folic concentration was highest in *G. Mosseae* treated soybean genotypes followed by *G. fasciculatum* and control. Significant enhancement of folic acid was obtained in soybean seeds upon VAM treatment is a beneficial feature from nutraceutical point of view. Nazim *et al.*[40] analyzed Vitamin B_{12} by UPLC-MS-MS in soy cheese and they reported 212.3723±9.80 µg folic acid on dry basis of soy chesses. Thus the enhancement of folic acid in VAM treated soybean plants would have implications in the soya based food products as well.

Influence of VAM on rutin content: The HPLC chromatogram of rutin and isoflavones compounds present in soybean seeds is shown in Fig. 3. Rutin content of VAM treated and control plant of soybean cultivar JS-335 was found to be 0.24% for control 0.41% for *G. fasciculatum* and 0.81% for *G. Mosseae* (Table 10). Among these highest percentage of rutin was found in *G. mosseae* treated soybean genotypes compared to *G. fasciculatum* and control. To current knowledge there are limited studies on rutin content in soybean. Magarelli *et al.*[41] reported that rutin and total isoflavone determination in soybean at different growth stages and they have found rutin concentration and total isoflavones in leaves, seeds and pods ranging from 0.44-1.7 mg g^{-1} and 72-128 µg g^{-1}, respectively. In comparison to this study the results which obtained was over 10 folds higher value, thus adding significant value to the crop by VAM treatments.

FTIR: Infrared spectroscopy involves the absorption of electromagnetic radiation in the infrared region of the spectrum. By analyzing sample in FTIR showed almost similar peaks (Fig. 4) when compared with standard (Fig. 5). Based on result, the samples were identified to contain Genistein and Daidzein.

Influence of VAM on isoflavone content: The HPLC chromatogram of the isoflavone compounds present in soybean seeds and of standard Daidzein and Genistein is shown in Fig. 5. Isoflavone content of the VAM treated and control plant of soybean cultivar JS-335 as analyzed by HPLC and quantity of Daidzein and Genistein in treated and non-treated soybean plants were presented in Table 11. This study found that VAM treatment has beneficially effect on soybean crop with high nutritional value.

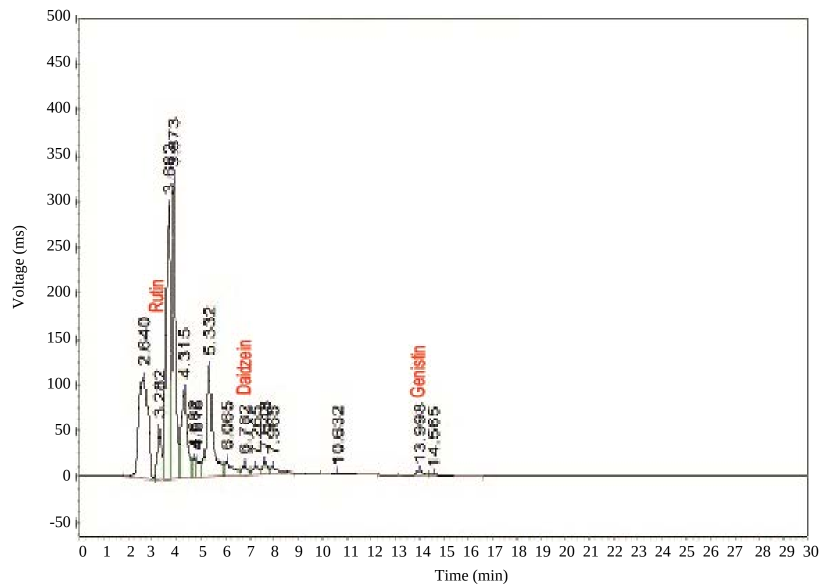

Fig. 3: FTIR spectra of isoflavone standard Daidzein FTIR spectra of isoflavone standard Genistein

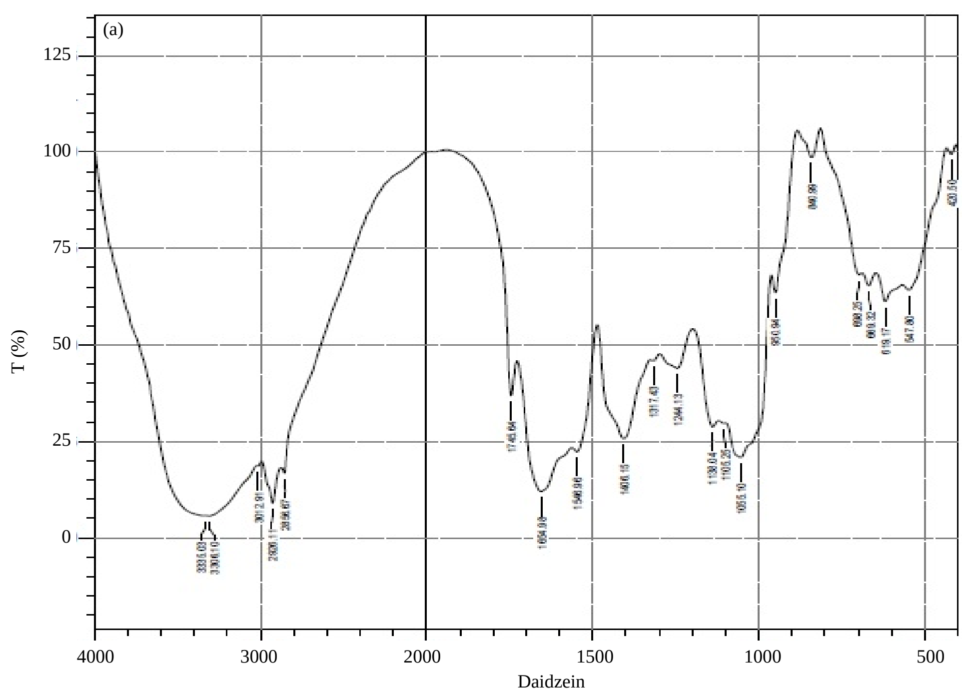

Fig. 4(a-b): Continue

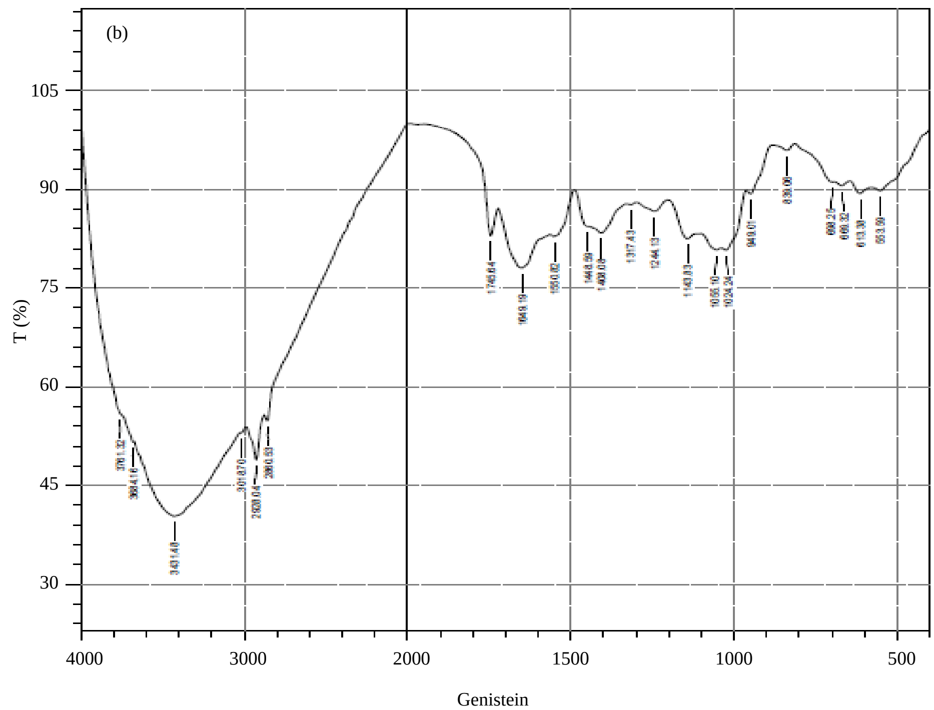

Fig. 4(a-b): FTIR spectra of Daidzein from the extract of soybean Genistein from the extract of soybean

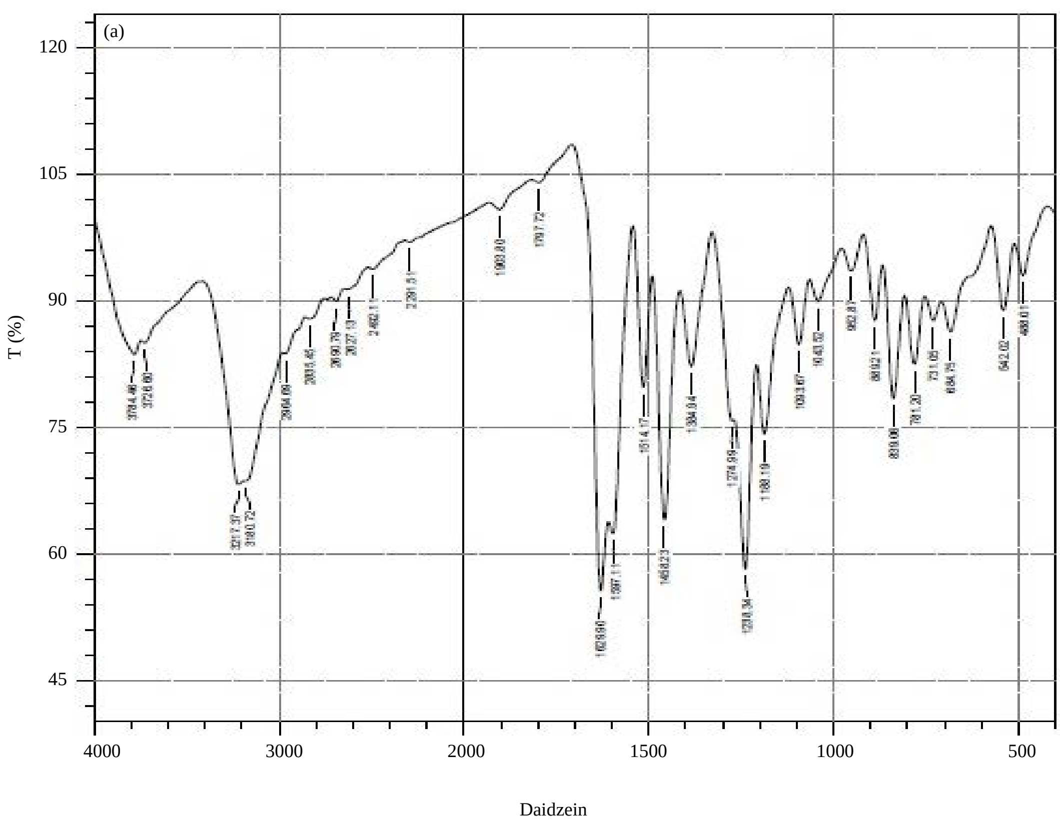

Fig. 5(a-b): Continue

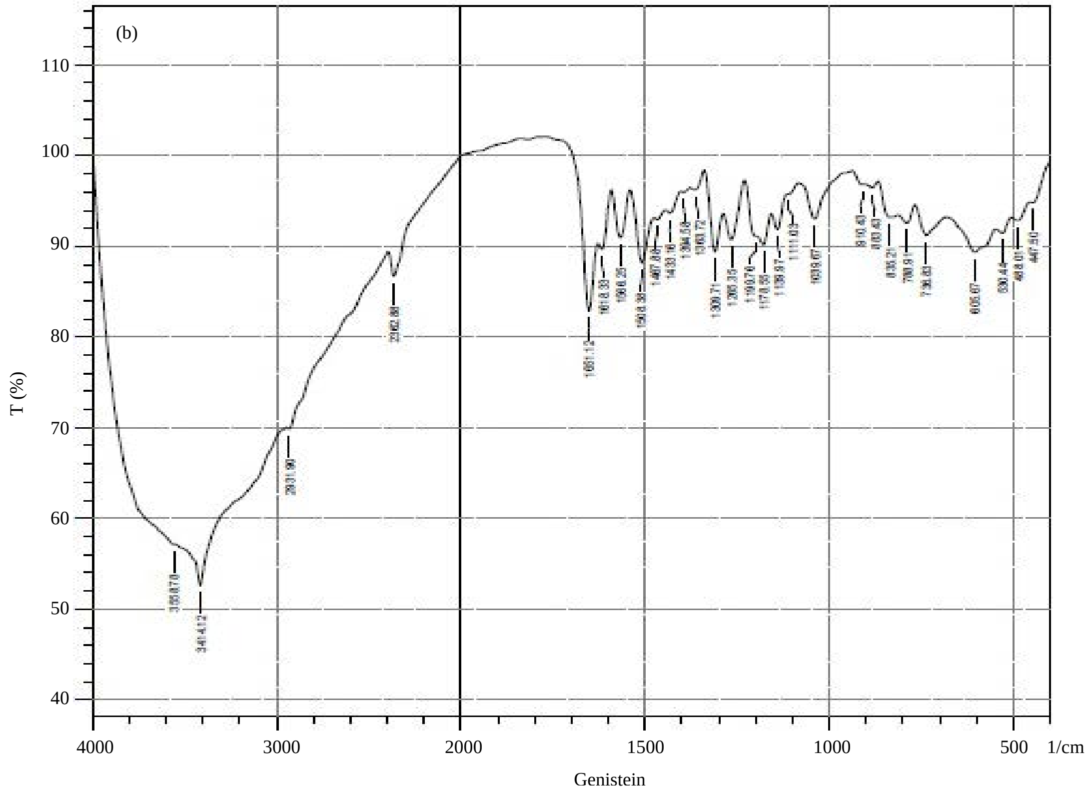

Fig. 5(a-b): HPLC chromatogram of isoflavone compounds in soybean seeds of JS-335

Table 11: Isoflavone concentration of control and VAM treated soybean seeds of cv.JS 335 (Dry weight basis)

	Isoflavone (µg g^{-1}) dry weight of seeds	
Treatments	Daidzein	Genistein
*Control	6.07±0.81	0.78±0.25
G. fasiculatum	19.50±1.24	5.77±1.06
G. mosseae	14.57±1.99	6.74±1.24

*Control: Without treatment of VAM,±standard deviation, $p<0.005$

CONCLUSION

In the present study seeds of soybean showed the presence of bioactive compounds such as flavonoid, folic acid, phenolic, isoflavone and antioxidants. Isoflavone profiling of soybean seeds will help to select suitable cultivars for their utilization in the preparation of processed soy products, thereby enhancing the health benefits of soy and soy-based foods. These results suggested that VAM treatment improves growth characteristics of soybean plant with an enhancement of antioxidant activity with concomitant increase in phenolic content, isoflavone, folic acid and flavonoid in seeds of VAM treated plants.

SIGNIFICANCE STATEMENT

The present study gave insights into the beneficial effect of Vesicular-arbuscular mycorrhizal (VAM) fungal for increased yield by enhanced growth and productivity. How are, a recent study explored enhancement of nutraceutical value was determined by measuring the total phenolic content and concentration of flavonoids in soybean as well as examining the rutin content, antioxidant activity folic acid content and isoflavones of soybean seeds in VAM treated plants. Therefore, the significant finding of this study could add to the knowledge regarding plant-fungi response to improvement of soybean crop productivity and nutraceutical point of view.

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