Effect of quizalofop and fenoxaprop on nutrient and antinutrient contents during seed development of mung bean (Vigna radiate L.)

Kumari Namrata, Jhuma Datta, Rajeev Kumar, A Chakravarty, Srikumar Pal and Nurnabi Meherul Alam

Abstract
In an effort to examine the impact of two herbicides viz. Quizalofop, and Fenoxaprop on the changes in contents of total phenol, sugar, starch and protein including trypsin inhibitory activity during the period of seed development of mung bean, a field experiment was conducted with application of these herbicides at their recommended field dose (RFD) and double the recommended field dose (dRFD) on 20 and 40 days after sowing of mung bean along with untreated control. The chemical analyses indicated that the untreated control followed by quizalofop treatment at RFD registered higher sugar, starch, protein and phenol content. On the other hand, diminished TIA level and enhanced antioxidant activity under DPPH assay were observed with untreated control followed by quizalofop treatment at dRFD. The rank order of different herbicide treatments in terms of antioxidant activity measured under different system was not similar. Based on the result, application of quizalofop at RFD can safely be recommended in mung bean cultivation.

Keywords: Mungbean, quizalofop, fenoxaprop, nutrient, antinutrient

Introduction
Mung bean (Vigna radiate L.) alternatively known as green gram, belonging to the family Fabaceae, is one of the leading pulse crop grown in India. Mung bean that holds key position, has established itself as highly valuable short duration crop having many desirable characters like high protein content (22-28%) (Abdel-Lateef, 1996; Abd El-Sattar et al., 2000)\(^1\), wider adaptability, low input requirement and ability to improve soil fertility. Besides having high protein content, mung bean seed is a rich source of carbohydrate (60-65%), vitamin C (Ghanem & Abbas, 2009)\(^1\) and essential amino acid, lysine, which is comparable to that of soybean (Glycine max L. Merr.) and kidney bean (Phaseolus vulgaris L) (Abdel-Lateef, 1996; Abd El-Sattar et al., 2000)\(^1\). In addition, during the course of its growth, mung bean produces several secondary metabolites viz. phenolic acids (benzoic and cinnamic acid derivatives), flavanoids, stilbenes, tannins and lignans which occur either in soluble free, soluble bound and insoluble bound (Kim et al. 2012, Tang et al. 2014)\(^1\) and essential amino acid, lysine, which is comparable to that of soybean (Glycine max L. Merr.) and kidney bean (Phaseolus vulgaris L) (Abdel-Lateef, 1996; Abd El-Sattar et al., 2000)\(^1\). In addition, during the course of its growth, mung bean produces several secondary metabolites viz. phenolic acids (benzoic and cinnamic acid derivatives), flavanoids, stilbenes, tannins and lignans which occur either in soluble free, soluble bound and insoluble bound (Kim et al. 2012, Tang et al. 2014)\(^1\). Thus, mung bean seed find its use as traditional and industrial food (Tang et al. 2014)\(^4\). However, the major constraint in the mung bean protein digestibility is the presence of antinutrient, trypsin inhibitor (Guillomen et al., 2008)\(^1\) that interferes with the availability of amino acids and pose serious concern to grazing animals and consumed uncooked (Bradbury and Holloway, 1988)\(^1\).

Weed infestation is one of the major factors limiting growth and productivity of mungbean. Applications of pre-emergence herbicides make it impossible to spray as this crop is grown during the rainy season. Moreover, weeds also emerge at a later stage, which necessitates the use of post-emergence herbicides such as. fenoxaprop-p-ethyl and quizalofop-ethyl as recommended by All India Network Programme (AIPN). Quizalofp and fenoxaprop are members of aryloxyphenoxypropionate family of herbicides, which exert toxicity by inhibiting acetyl CoA carboxylase (ACCase), which catalyzes the committed step in de novo fatty acid biosynthesis (Burton, 1989; Focke & Lichtenthaler, 1987)\(^8\), leading to impairment of biosynthesis of lipids (Stoltenberg, 1989)\(^3\). Herbicides, in addition to their recognized role, have also been shown to modulate the activities of various enzymes of primary and secondary metabolism. Several herbicides have been reported to modulate either positively or negatively the phenol content of various crops by activating or inhibiting the enzymes of phenol synthesis (Namrata et al., 2020)\(^1\).
Similarly a number of herbicides affect the enzymes of nitrogen cycle (nitrate reductase (NR), nitrite reductase (NiR), glutamine synthetase and glutamate 2-oxoglutarate amino transferase (Miflin et al., 1990, Lea et al., 1990 & Goodwin et al., 1983) [22, 21, 19], which adversely affects protein content as well as yield. Thus herbicides, by virtue of their profound influence on the enzymes of phenol, carbohydrate and amino acid metabolism, can modulate the content of these phytochemicals. With these background information, an attempt has been made in present study to find out the impact of two herbicides viz. Quizalofop, and Fenoxaprop, each applied at their recommended and double the recommended dose, on the changes in the content of phenol (total and free phenol), carbohydrate (starch and sugar), protein and trypsin inhibitory activity during the period of seed development of mung bean. Herbicide induced modulation of antioxidant activity of phenol extracts using different assay systems viz. DPPH, ABTS and FRAP is also examined.

Material and methods

Plant material and chemical
A field experiment was conducted at the University research farm, Bidhan Chandra Krishi Vishwavidyalaya, Mohanpur, Nadia, West Bengal using two post emergent herbicide viz, quizalofop and fenoxaprop. Mung bean were raised after washing and sterilization of seeds with 0.5% of HgCl₂ (w/v) for 10 minutes followed by washing with distilled water thrice to remove the traces of HgCl₂. Herbicides were applied at their recommended field dose (RFD) and double the RFD (dRFD). Quizalofop @ 37.5 kg ai/ha (RFD) and 75 kg ai/ha (dRFD), while fenoxaprop @ 50 kg ai/ha (RFD) and 100 kg ai/ha (dRFD) were applied on 20 and 40 days after sowing. Each treatment was replicated thrice.

Sampling
Seed samples of mung bean were collected from each treatment replications periodically at 10, 15, 20, 25 and 30 days after fruit setting (DAFS). Seed samples were oven dried at 40 °C until constant weight and ground using an electric grinder. The dried sample was then subjected to chemical analysis.

Chemical
ABTS was procured from Fluka Chemical Co. and Trolox from Sigma Aldrich Chemical Co. Other reagents were of analytical grade.

Chemical analysis

Analysis of total sugar and starch
Sugar and starch were extracted in 15 ml of 80% anhydrous alcohol by boiling 0.1 g dry powdered sample for 30 min at 80 °C followed by centrifugation at 10,000 rpm for 30 minute. Alcohol was evaporated off in a water bath at 80 °C and made to a volume of suitable volume with water and used as sugar extract. Following sugar extraction, the residue after oven drying at 80 °C was used for starch extraction using 52% perchloric acid at 80 °C for 20 min. Starch extract was obtained following centrifugation at 10,000 rpm for 30 minute. 0.5 ml and 0.2 ml of both the extracts was taken in separate test tubes after proper dilution and the final volume was adjusted to 1 ml with distilled water. Subsequent analysis was performed as per method described by Sen et al., (2005) [36]. The amount of sugar and starch was estimated from the standard curve, which was prepared using a series of standard glucose solutions between 0 and 100 µg/ml.

Estimation of Crude Protein
The crude protein content was measured based on the analysis of total nitrogen (N) content using Kjeldahl method (Sadasivam and Manikam, 2011) [34]. The samples were digested taking 0.5g dried sample, 10g of digestion mixture (K2SO4:FeSO4:CuSO4:5H2O in 10:1:1 ratio) and 15 ml of 0.1 N concentrated Sulphuric acid for 2 hours when green colour was developed. The solution was cooled and 15 ml of water and 70 ml of 40 per cent NaOH was added and volatile ammonia was collected by distillation in 250 ml conical flask containing 25 ml of 4 % boric acid. The nitrogen content was measured by titrating against 1 % sulphuric acid. At the end point colour changed from green to pink. Amount of nitrogen in the samples was calculated by the following relation.

% of nitrogen = (T-B) x1.4 x N / W

Where T and B refers to amount of sulphuric acid consumed for test and blank sample, while N and W represents the strength of sulphuric acid and weight of the sample respectively.

Trypsin inhibitor (EC 3.4.21.4) analysis
0.1 g dried sample was homogenized for 2 minutes with 10 ml Tris - CaCl2 buffer solution (0.04 M Tris, 0.01 M CaCl2 and water, pH adjusted to 8.1). The homogenate was allowed to stand for 5 minutes before centrifugation at 10,000 rpm at 5 °C (Bradburry and Hammer, 1990) [6]. Aliquot of the supernatant was diluted four times with the buffer solution (pH 8.1) to give a range of 4 or 5 solutions of different concentrations of TI extract. The trypsin inhibitor (TI) activity was assessed by incubating 50 µl of crude extract of TI with 20 µl of commercial bovine trypsin (1 mg mL⁻¹) at 37 °C for 15 min, following the method adopted by Kakade et al. (1974) [18]. Then, 40µl (from the stock solution of 10 mg mL⁻¹ in Dimethyl Sulfoxide) BApNA (N-a benzal-DL-Arginine p-nitro anilide) was added to the assay solution and the mixture was again incubated at 37 °C for 30 min. Reaction was stopped by adding 0.5 ml of 30 % of glacial acetic acid and the absorbance of the reaction mixture was measured at 410 nm against a blank without substrate and a blank containing crude extract without BApNA in order to subtract the absorbance of the crude extract. Trypsin inhibitory activity (TIA) was determined by the difference between the enzyme activity in the absence and in the presence of inhibitors. One TIU is defined as a decrease in A₄₁₀ by 0.01 in 10 minutes. TIA is expressed in the units of trypsin inhibited (TIU) per mg of dry matter of the sample.

Total Phenol
The total phenol content in mung bean seed was determined using FolinCiocalteau Reagent (FCR) (Vinson et al., 1998) [41]. 0.1g Dried and pulverized mung bean powder was extracted with 15 ml of 1.2 N HCl in 50% aqueous methanol by shaking in water bath at 90 °C for 2 hours to determine conjugated plus un-conjugated (‘total’) phenol. The solution was centrifuged at 10,000 rpm for 30 minutes. The supernatant was evaporated to dryness and suitably diluted and subsequent analysis was followed using FCR and the absorbance was recorded at 650 nm. Gallic acid was used as a standard for comparison and the results were expressed in milligrams of Gallic Acid Equivalent per gram dry matter (mg GAE/g DM).
Antioxidant activity
Antioxidant activity of the total phenolic extract was examined using DPPH and ABTS radical according to method described by Braca et al., (2001) [5] and Ozgen et al., (2006) [27] respectively, while FRAP assay using the method adopted by Benzie and Strain (1996). Briefly, in each assay technique, 150 µL of sample was added with 2850 µL of respective assay solution and kept in dark for 30 min. Finally, the change in absorbance with or without extract was read at 517 nm, 734 nm and 593 nm for DPPH, ABTS and FRAP assay respectively. The antioxidant activity was expressed in mg trolox equivalent per g (mg TE/g).

Statistical analysis
All data were subjected to analysis statistically by ANOVA of a RBD design, to determine differences among means. Statistical analyses were done using SPSS Professional Statistics ver. 7.5 (SPSS Inc., Irvine, California).

Results and Discussion
Effect of herbicides on sugar and starch content of mung bean
The comparison of mean sugar content in response to different treatments over different sampling days indicated that untreated control and fenoxaprop at dRFD registered significantly higher and lower sugar content respectively, while other treatments showed no significant differences in their sugar content. Similar to sugar, the starch content, although higher for untreated, was comparable for treatments with quizalofop and fenoxaprop at their RFD. On the other hand, significant increase in mean sugar content over different treatments was observed up to 20 DAFS, which finally declined, while the corresponding figure for starch content showed no significant differences during this period. However, both starch and sugar content reached maximum on 25 DAFS. The results thus indicated that quizalofop application at dRFD reduced the sugar accumulation, while application of both quizalofop and fenoxaprop at RFD. The range of values of starch content in mung bean seed obtained in the present study compared well with earlier report (Elkovicz et al., 1982 & Shi et al., 2016) [10, 37]. Moreover, the interaction between treatment and sampling days for these chemical components were significant.

Effect of herbicides on protein content and trypsin inhibitory activity of mung bean
Mung bean, an important source of protein and energy, form an important part of human diet and animal feed in many parts of the world. Among several anti-nutritional factors, the most important is trypsin inhibitor that can inactivate the digestive enzymes when reach the small intestine unaltered (Belitz et al., 1982) [4]. The effect of herbicides on protein and trypsin inhibitory activity (TIA) during seed development of mung bean has been summarized in Table 2, which indicated that there is no significant variation in protein content between the treatments such as untreated control and herbicides treatment at RFD. The protein content in mung bean varied from 17.65% to 20.65% which is considerably low as compared to earlier report (Ofuya et al., 2005) [26]. This difference probably results from differences in genotype and growing condition. Moreover, the mean protein content of mung bean increased significantly up to 20 DAFS and then declined. Similar trend in protein content was also observed with all the treatments. On the other hand, fenoxaprop at the dRFD resulted a maximum increase in TIA (722.48mg TIU/g DM) over control (635.34TIU/g DM). The mean TI content of mung bean increased significantly up to 25 DAFS and then declined. It was also evident that the mean TIA fluctuated during the seed development. This similar trend was also noticed during the seed development in kidney bean (Alizadeh et al., 2012) [3]. Moreover, the relationship between protein and TIA, although positive, but was not significant. TIA level observed in the present study was found to be within the range as reported by Rasha et al., (2011) [29]. The increase in TIA over control due to herbicide treatment, which was more pronounced with Fenoxaprop higher dose, is linked to herbicide-induced oxidative stress (Mondy and Chandra, 1979, Ryan, 1973) [23, 39]. Thus, it appears that both these herbicides at their dRFD affect protein and TIA level by their profound influence on activities of acetolactate synthase, glutamine synthetase and 2oxoglutarate by Glutamate 2-oxoglutarate amino transferase (Lea et al., 1990) [21].
mung bean extracts in different testing system may also affect the capacity of individual extracts to react or quench different radicals. Secondary metabolites are responsible for the antioxidant activity of fruits, and variations during the stages of development are related to the physiological maturation of fruits (Taiz & Zeiger, 2006) [27] or even in response to factors such as physiological stage of the crop (Garcia et al., 2019) [13] and environmental stresses, which involve temperature, oxygen, and pathogens (Roussos et al., 2007) [31].

Table 1: Changes in sugar and starch content (%) at different days after fruit setting (DAFS) of mung bean (*Vigna radiata L.*).

<table>
<thead>
<tr>
<th>Treatments (ai g ha⁻¹)</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀ (control)</td>
<td>49.99⁺</td>
<td>46.85³</td>
<td>46.85³</td>
<td>65.96⁵</td>
<td>62.59⁶</td>
<td>54.45⁷</td>
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<tr>
<td>T₁ (Quizalofop 37.5)</td>
<td>53.12⁺</td>
<td>56.93⁵</td>
<td>52.98⁶</td>
<td>61.78⁴</td>
<td>54.12⁷</td>
<td>57.79⁸</td>
</tr>
<tr>
<td>T₂ (Quizalofop 75)</td>
<td>46.75⁺</td>
<td>49.89⁷</td>
<td>45.09⁺</td>
<td>57.1⁺</td>
<td>52.17⁺</td>
<td>50.2⁺</td>
</tr>
<tr>
<td>T₃ (Fenoxaprop 50)</td>
<td>53.61⁺</td>
<td>54.1⁺</td>
<td>50.4⁺</td>
<td>54.87⁺</td>
<td>53.6⁺</td>
<td>54.1⁺</td>
</tr>
<tr>
<td>T₄ (Fenoxaprop 100)</td>
<td>49.25⁺</td>
<td>46.38⁺</td>
<td>52.28⁺</td>
<td>53.17⁺</td>
<td>46.33⁺</td>
<td>49.48⁺</td>
</tr>
<tr>
<td>Total Mean</td>
<td>50.55⁺</td>
<td>50.29⁺</td>
<td>50.85⁺</td>
<td>58.58⁺</td>
<td>53.76⁺</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation of triplicate samples. Same English Letter followed by mean are not significantly different in a column and same Greek letter followed by mean are not significantly different within a row.

Table 2: Changes in protein content (%) and TIA level (IU/mg DM) at different days after fruit setting (DAFS).

<table>
<thead>
<tr>
<th>Treatments (ai g ha⁻¹)</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀ (control)</td>
<td>17.65⁺</td>
<td>20.36⁺</td>
<td>20.65⁺</td>
<td>20.42⁺</td>
<td>18.35⁺</td>
<td>19.49⁺</td>
</tr>
<tr>
<td>T₁ (Quizalofop 37.5)</td>
<td>19.7⁺</td>
<td>19.89⁺</td>
<td>20.83⁺</td>
<td>20.08⁺</td>
<td>18.88⁺</td>
<td>19.88⁺</td>
</tr>
<tr>
<td>T₂ (Quizalofop 75)</td>
<td>19.09⁺</td>
<td>19.36⁺</td>
<td>20.14⁺</td>
<td>19.28⁺</td>
<td>18.81⁺</td>
<td>19.34⁺</td>
</tr>
<tr>
<td>T₃ (Fenoxaprop 50)</td>
<td>20.05⁺</td>
<td>20.26⁺</td>
<td>21.0⁺</td>
<td>19.29⁺</td>
<td>18.62⁺</td>
<td>19.84⁺</td>
</tr>
<tr>
<td>T₄ (Fenoxaprop 100)</td>
<td>19.26⁺</td>
<td>19.53⁺</td>
<td>19.72⁺</td>
<td>18.03⁺</td>
<td>18.11⁺</td>
<td>18.93⁺</td>
</tr>
<tr>
<td>Total Mean</td>
<td>19.15⁺</td>
<td>19.88⁺</td>
<td>20.47⁺</td>
<td>19.47⁺</td>
<td>18.55⁺</td>
<td></td>
</tr>
</tbody>
</table>

Tukey’s HSD for treatment × DAF (p<0.05) = 0.28

Data are expressed as mean ± standard deviation of triplicate samples. Same English Letter followed by mean are not significantly different in a column and same Greek letter followed by mean are not significantly different within a row.

Table 3: Changes in total phenol content (mg GAE/g DM) and Antioxidant activity under DPPH (mg TE/g DM) assay at different days after fruit setting (DAFS).

<table>
<thead>
<tr>
<th>Treatments (ai g ha⁻¹)</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀ (control)</td>
<td>47.24⁺</td>
<td>42.73⁺</td>
<td>38.13⁺</td>
<td>35.74⁺</td>
<td>31.02⁺</td>
<td>38.97⁺</td>
</tr>
<tr>
<td>T₁ (Quizalofop 37.5)</td>
<td>48.86⁺</td>
<td>36.48⁺</td>
<td>30.42⁺</td>
<td>29.95⁺</td>
<td>29.68⁺</td>
<td>35.08⁺</td>
</tr>
<tr>
<td>T₂ (Quizalofop 75)</td>
<td>44.52⁺</td>
<td>30.11⁺</td>
<td>29.53⁺</td>
<td>29.92⁺</td>
<td>28.63⁺</td>
<td>32.54⁺</td>
</tr>
<tr>
<td>T₃ (Fenoxaprop 50)</td>
<td>37.36⁺</td>
<td>31.14⁺</td>
<td>27.91⁺</td>
<td>23.12⁺</td>
<td>21.86⁺</td>
<td>28.28⁺</td>
</tr>
<tr>
<td>T₄ (Fenoxaprop 100)</td>
<td>34.47⁺</td>
<td>30.01⁺</td>
<td>27.06⁺</td>
<td>21.83⁺</td>
<td>16.91⁺</td>
<td>26.06⁺</td>
</tr>
<tr>
<td>Total Mean</td>
<td>42.49⁺</td>
<td>34.10⁺</td>
<td>30.62⁺</td>
<td>28.11⁺</td>
<td>25.62⁺</td>
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</tr>
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</table>

Tukey’s HSD for treatment × DAF (p<0.05) = 0.35

Data are expressed as mean ± standard deviation of triplicate samples. Same English Letter followed by mean are not significantly different in a column and same Greek letter followed by mean are not significantly different within a row.

Table 4: Changes in Antioxidant activity under ABTS and FRAP (mg TE/g DM) assay at different days after fruit setting (DAFS).

<table>
<thead>
<tr>
<th>Treatments (ai g ha⁻¹)</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
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<td>4.76⁺</td>
<td>4.46⁺</td>
<td>6.61⁺</td>
<td>4.75⁺</td>
<td>4.96⁺</td>
</tr>
<tr>
<td>T₁ (Quizalofop 37.5)</td>
<td>4.58⁺</td>
<td>3.33⁺</td>
<td>3.52⁺</td>
<td>3.71⁺</td>
<td>3.35⁺</td>
<td>3.70⁺</td>
</tr>
<tr>
<td>T₂ (Quizalofop 75)</td>
<td>4.72⁺</td>
<td>3.54⁺</td>
<td>3.68⁺</td>
<td>4.11⁺</td>
<td>3.54⁺</td>
<td>3.92⁺</td>
</tr>
<tr>
<td>T₃ (Fenoxaprop 50)</td>
<td>3.47⁺</td>
<td>3.54⁺</td>
<td>4.32⁺</td>
<td>4.08⁺</td>
<td>3.54⁺</td>
<td>3.79⁺</td>
</tr>
<tr>
<td>T₄ (Fenoxaprop 100)</td>
<td>4.24⁺</td>
<td>3.79⁺</td>
<td>4.8⁺</td>
<td>4.37⁺</td>
<td>3.78⁺</td>
<td>4.20⁺</td>
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<tr>
<td>Total Mean</td>
<td>4.25⁺</td>
<td>3.79⁺</td>
<td>4.16⁺</td>
<td>4.58⁺</td>
<td>3.79⁺</td>
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</tr>
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</table>

Tukey’s HSD for treatment × DAF (p<0.05) = 0.21

Data are expressed as mean ± standard deviation of triplicate samples. Same English Letter followed by mean are not significantly different in a column and same Greek letter followed by mean are not significantly different within a row.

Conclusion

Based on all chemical parameters analyzed in the present study, it may be concluded that quizalofop treatment at RFD appeared to be promising treatment without any adverse effect on carbohydrate, protein and phenol content, while fenoxaprop treatment at DRFD seemed to be a better option in reducing TIA level and enhancing AOA under DPPH assay. Thus, application of quizalofop at lower application rate can safely be recommended in mung bean cultivation.

References

2. Abd El-Sattar AM, Shaaban MM, El-masy MF. Yield potential and nutrients uptake and utilization of some


