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Impact of weed management and phosphorus application on nutrient content, uptake and biochemical parameters of cowpea [*Vigna unguiculata* (L.) Walp.]

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Abstract
A field experimentation was undertaken during *Kharif* 2019 at Instructional Farm of Agronomy, Rajasthan College of Agriculture, and Udaipur to study the influence of weed management and phosphorus fertilization on nutrient content, uptake and biochemical parameters of cowpea (*Vigna unguiculata* (L.) Walp.). Outcome of this research revealed that maximum chlorophyll content, seed protein content, nitrogen and phosphorus content of seed and haulm and their uptake by seed, haulm and total uptake by cowpea crop were recorded with pre-emergence application of pendimethalin 750 g ha^{-1} in combination with imazethapyr + imazamox 33.75 g ha^{-1} as post-emergence at 15-20 DAS next to weed free treatment. Whereas, application of phosphorus at various doses influenced chlorophyll content, seed protein content, nitrogen and phosphorus content of seed and haulm and their uptake by seed, haulm and total uptake by cowpea crop significantly.

Keywords: Chlorophyll content, protein content, haulm, nitrogen, phosphorus, nutrient uptake

Introduction
Cowpea is one of the important legumes in the world which plays an important role in the livelihood of millions of people in developing countries (El Naim and Jabereldar, 2010) [1]. As it is a warm season crop, it is grown throughout the semi-arid regions of the tropics and sub-tropics. It is known for its versatility as it is used for grain, leaf and forage, cover crop and green manure crop. Being a leguminous pulse, it has high nutritive value and high palatability (Whitebread and Lawrence, 2006) [2]. Because of its nitrogen fixing ability, it is known for improving soil fertility (Abayomi and Abidoye, 2009) [3]. It can be consumed in all stages of its growth and can be used to prepare delicious dishes and animal feed. It is a popular *Kharif* as well as summer pulse crop. It is well-known for its smothering nature, drought tolerant character, soil restoring properties and multiple uses. It is also known as "vegetable meat", because it is a rich source of protein and other nutrients and minerals like calcium and iron. On dry weight basis, cowpea seeds contain 23.4 per cent protein, 1.8 per cent fat, 60.3 per cent carbohydrate, 3.4 per cent fibre, 3.3 per cent ash and 9 to 11 per cent moisture. The world's estimated annual cowpea production is put at 4.5 million tonnes from an estimated land area of 12.6 million hectares (FAO, 2014) [4]. In India, it is grown on about 0.5 million ha land area with an average productivity of 750 kg seeds ha^{-1} (Anonymous, 2017) [5]. Whereas, in Rajasthan this crop occupied 88,592 hectares with production of 64,802 tonnes and productivity of 731 kg ha^{-1} (Anonymous, 2016) [6]. The productivity of the crop in this zone i.e., Zone-IV (a) of Rajasthan is low and far below than its potential yield, possibly due to inadequate weed management as well as poor nutrition especially phosphorus fertilization.

During rainy season, the crop suffers severely due to weed infestation resulting into wide range reduction in crop yield. The critical period of crop weed competition in cowpea has been identified as 15-25 days after sowing depending on the varieties and other factors; presence of weeds beyond this period causes severe reduction in yield. Hence, weed control needs to be undertaken during initial period of crop growth. Although hoeing and weeding is a traditional yet effective method of weed control but non-availability of labors at peak period as well as high Labour cost and un-workable soil conditions are the main limitations of manual weeding. Under such situation the only alternative left before the farmers is to use suitable herbicides. However, the herbicidal weed control in cowpea is limited due to the scarcity of studies pertinent to the selectivity of herbicides for this crop.

There are many researches on herbicide usage in legumes, but in cowpea, very meagre research has been done in this zone. For weed control in cowpea, only pendimethalin is the most popular among all the available herbicides which is applied either alone or in combination with hand weeding. There are no specific recommendations on the use of new herbicide molecules available in mixture formulations in this zone. Looking to these facts, it is planned to evaluate the performance of new herbicide molecule imazethapyr + imazamox as post-emergence alone and in sequence after pendimethalin in order to explore the possibility of better weed management option for this crop.

Legumes show good response to phosphorus application owing to their positive influence on root growth, nodule development, bacterial activity and nitrogen fixation. Root development, vigour of the plant, flower and seed formation, crop maturity and resistance to pests and diseases are the attributes associated with phosphorus availability. Phosphorus is very much required to enhance cowpea yield because it stimulates growth, initiate nodules formation as well as influencing the efficiency of rhizobium-legume symbiosis (Namakka *et al*., 2017) [7]. Phosphorus has beneficial influence on both nodulation and enhancing seed yield of cowpea (Singh *et al*., 2011) [8]. The factor productivity of crops in general is declining under intensive cropping system. Therefore, to enhance the yield of crops in general and cowpea in particular, more nutrients particularly phosphorus is required to be added. It is an important nutrient for plant growth but its availability is often limited. Phosphorus deficiency also indirectly reduces nitrogen supply to legumes by affecting nitrogen fixation. Both abiotic and biotic factors limit its cultivation and production. The most important abiotic factor is the soil related constraints i.e., low soil fertility which is the most severe on more than half of the arable land in the tropics. Hence, this study was conducted to investigate the influence of weed management and phosphorus fertilization on nutrient content, uptake and biochemical parameters of cowpea (*Vigna unguiculata* (L.) Walp.).

Materials and Methods

Experimental site and soil

The experiment was conducted at the Instructional Farm, Rajasthan College of Agriculture, and Udaipur. The experimental plot is located at South-Eastern part of Rajasthan at an altitude of 582.17 meter above mean sea level and at 24˚35′ N latitude and 73˚42′ E longitude. The region comes under agro-climatic zone IVa *i.e.* "Sub-Humid Southern Plain and Aravalli Hills" of Rajasthan. The texture of soil of experimental field was clay loam and it was slightly alkaline in reaction. It was medium in available nitrogen (288 kg ha^{-1}), phosphorus (20.54 kg ha^{-1}) and high in potassium (286.92 kg ha^{-1}). The total rainfall received during the crop season was 729.6 mm.

Experimental design and treatments

The experiment was laid out in factorial randomized block design with three replications and containing 18 treatment combinations consisting of 6 weed management treatments (Pendimethalin 1000 g ha^{-1} PE *fb* hoeing and weeding 15-20 DAS, imazamox + imazethapyr 45 g ha^{-1} at 15-20 DAS, pendimethalin 750g ha^{-1} PE *fb* imazamox + imazethapyr 33.75 g ha^{-1} at15-20 DAS, one hoeing and weeding 15-20 DAS, weed free up to 50 days and weedy check) and 3 phosphorus levels (30, 40 and 50 kg P_2O_5 ha^{-1}). As per treatments, the needed quantities of fertilizers were applied below the seed at the time of sowing. Cowpea variety RC-101 was used as test crop with recommended package of practices. After seed treatment with fungicide (Bavistin 2 g kg^{-1} seed), cowpea seeds were sown in opened furrows at the rate of 15 kg seeds ha^{-1} with 30 cm row to row spacing and 10 cm plant to plant spacing ad seeds were sown at a depth of 3-4 cm. Seeds were covered with soil after sowing.

Weed management

As per treatment, pendimethalin was applied one day after the sowing of crop when there was sufficient moisture in the soil, while application of imazethapyr + imazamox and hoeing and weeding were done at 15-20 DAS. These herbicides were sprayed using 700 liters of water ha^{-1} with knapsack sprayer fitted with flat fan nozzle after calibration. Hand weeding was done with hand hoe.

Table 1: Details of Herbicides used

S. No.	Common Name	Trade Name	Chemical name
1.	Pendimethalin	Pendamil	N-(1-ethylproyl)-3,4-dimethyl-2, 6-dinitrobenzenamine
2.	Imazethapyr + imazamox	Odessey	5-ethyl-2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]nicotinic acid + 2-[(RS)-4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl]-5-methoxymethylnicotinic acid

Biochemical studies:

(I) Chlorophyll content (mg g^{-1} fresh weight of leaves)

For analysis of total chlorophyll content fresh leaf samples were collected from each experimental plot at 50 DAS early in the morning and the same samples were crushed in pestle and mortar with 80% acetone, supernatant from all the samples were collected and final volume of the extract was made to 10 ml with 80% acetone and optical density of all the samples were measured using spectrophotometer at 652 nm and total chlorophyll content was calculated by the following formula using procedure laid down by (Arnon, 1949) using 80% acetone.

$$\text{Total chlorophyll content}\,(mg/g) = \frac{\text{OD at 652 nm}}{34.5} \times 1000 \times \frac{V}{1000 \times W}$$

Where, OD is optical density, W is weight of fresh leaf sample in grams and V is final volume of extract taken for optical density reading.

(II) Seed protein content (%)

Seed protein content was worked out by multiplying the per cent of total nitrogen in the seed with a common factor 6.25.
Seed protein content (%) = N content in seed (%) x 6.25.

(III) Nutrient content

Seed and haulm samples were collected at harvest from each experimental plot and were oven dried at 65˚C till a constant weight attained and grinded in a laboratory grinder. These samples were subjected to chemical analysis for determining N and P contents. The following standard methods were adopted for analysis.

Nitrogen	Nessler's reagent colorimetric method (Lindner, 1944) [10]
Phosphorus	Ammonium vanadomolybdate yellow colour method (Richards, 1968) [11]

(IV) Nutrient uptake

N and P uptake by seed and haulm at harvest were estimated by using following formula

$$\text{Nutrient uptake by seed / haulm} \left(\text{kg ha}^{-1}\right) = \frac{\text{Nutrient content in seed / haulm} (\%) \times \text{Seed / haulm yield (kg ha}^{-1})}{100}$$

Statistical analysis: The data recorded for evaluation of treatment was exposed to statistical analysis by applying technique of analysis of variance described by (Cochran and Cox, 1967) [12] in order to test the significance of the experimental results. Wherever, the "F" test was significant at 5 per cent level of significance, the critical differences (CD) for the treatments means was worked out.

Results and Discussion

Weed free treatment recorded maximum chlorophyll content (3.23 mg g^{-1}) followed by pre-emergence application of pendimethalin 750 g ha^{-1} in combination with post-emergence application of imazethapyr + imazamox 33.75 g ha^{-1} at 15-20 DAS (2.96 mg g^{-1}). The corresponding values of chlorophyll content under pendimethalin 1000 g ha^{-1} along with hoeing and weeding at 15-20 DAS and imazethapyr + imazamox 45 g ha^{-1} and one hoeing & weeding at 15-20 DAS were 2.92, 2.79 and 2.73 mg g^{-1} fresh weight of leaves, respectively. Weed free treatment was found to be superior in terms of seed protein content of cowpea (23.11%) which was statistically at parity with pendimethalin 750 g ha^{-1} as pre-emergence in conjugation with imazethapyr + imazamox 33.75 g ha^{-1} as post-emergence (22.84%). The respective values of seed protein content owing to pendimethalin 1000 g ha^{-1} along with hoeing and weeding at 15-20 DAS, imazethapyr + imazamox 45 g ha^{-1}, one hoeing & weeding at 15-20 DAS and control were 22.38, 22.18, 21.49 and 20.03 per cent. This result is analogous to those reported by Jha and Soni (2013) [13] and Yadav *et al*. (2017) [14].

Application of each successive doses of phosphorus consistently enhanced chlorophyll content of cowpea but the enhancement was at decreasing rate. The per cent increase in chlorophyll content g^{-1} of fresh weight of leaves at 50 DAS owing to 40 and 50 kg P_2O_5 ha^{-1} was 13.56 and 18.60, respectively in relation to 30 kg P_2O_5 ha^{-1}. This can be described based on the fact that nitrogen is a structural element of pay role rings of chlorophyll, phosphorus fertilization enhances biological nitrogen fixation by increasing effective root nodules $plant^{-1}$ which in turn augments chlorophyll content of leaf by its indirect effect on formation of chloroplast and accumulation of chlorophyll in them. This result coincides with the result of Jha *et al*. 2014 [15], but these authors used higher phosphorus doses than in the present investigation.

Addition of phosphorus at 40 kg P_2O_5 ha^{-1} (22.28 %) significantly augmented seed protein content of cowpea compared to 30 kg P_2O_5 ha^{-1} (21.00 %) and further addition of phosphorus by 10 kg failed to augment this parameter significantly. This might be attributed to the function of phosphorus in synthesis of nucleic acids; initiation of nodule formation besides influence on rhizobium-legume symbiosis thus increasing nitrogen enhances seed protein content of cowpea.

Table 2: Effect of weed management and phosphorus levels on chlorophyll content and seed protein content

Treatments	Chlorophyll content (mg g^{-1} fresh weight of leaves) 50 DAS	Seed protein content (%)
Weed management		
Pendimethalin 1000 g PE *fb* HW	2.92	22.38
Imazethapyr + imazamox 45 g	2.79	22.18
Pendimethalin 750 g PE *fb* imazethapyr + imazamox 33.75 g	2.96	22.84
One hoeing and weeding 15-20 DAS	2.73	21.49
Weed free (up to 50 days)	3.23	23.11
Weedy check	2.52	20.03
SEm±	0.03	0.16
CD(P= 0.05)	0.09	0.45
Phosphorus levels (P_2O_5 kg ha^{-1})		
30	2.58	21.00
40	2.93	22.28
50	3.06	22.74
SEm±	0.05	0.22
CD(P= 0.05)	0.13	0.64

Maximum nitrogen content of seed and haulm was documented with weed free treatment (3.70 and 0.979 per cent, respectively) which was statistically at par with pre-emergence application of pendimethalin 750 g ha^{-1} along with post-emergence application of imazethapyr + imazamox 33.75 g ha^{-1} (3.65 and 0.961 per cent, respectively) and minimum nitrogen content of seed and haulm was observed with weedy check (3.20 and 0.853 per cent, respectively). Weed free treatment resulted in maximum phosphorus content in seed and haulm (0.443 and 0.220 per cent, respectively) which was statistically at par with pendimethalin 750 g ha^{-1} as pre-emergence in conjugation with imazethapyr + imazamox 33.75 g ha^{-1} as post-emergence (0.437 and 0.217 per cent, respectively).

The biochemical analysis of plant parts at harvest elucidated that application of phosphorus at 40 kg P_2O_5 ha^{-1} substantially influenced N and P content and their uptake by seed, haulm and total uptake over 30 kg P_2O_5 ha^{-1} and further addition of phosphorus from 40 to 50 kg P_2O_5 ha^{-1} failed to enhance N uptake by seed, haulm and total uptake. The noticeable enhancement in these parameters appears to be because of its higher accessibility in soil to the plant with their higher removal by roots and boosted translocation in plants. These results were in tune with the research findings of Karche *et al.* (2012) [16]. This might be ascribed to direct effect of phosphorus nutrition and indirect effect of phosphorus on nodulation and nitrogen fixation thereby more N and P uptake by crop.

Table 3: Effect of weed management and phosphorus levels on N and P content (%) in seed and haulm of cowpea at harvest

Treatments	Nitrogen		Phosphorus	
	Seed	Haulm	Seed	Haulm
Weed management				
Pendimethalin 1000 g PE *fb* HW	3.58	0.953	0.428	0.210
Imazethapyr + imazamox 45 g	3.55	0.940	0.423	0.206
Pendimethalin 750 g PE *fb* imazethapyr + imazamox 33.75 g	3.65	0.961	0.437	0.217
One hoeing and weeding 15-20 DAS	3.44	0.929	0.408	0.197
Weed free (up to 50 days)	3.70	0.979	0.443	0.220
Weedy check	3.20	0.853	0.377	0.192
SEm±	0.03	0.007	0.003	0.002
CD(P= 0.05)	0.07	0.020	0.010	0.007
Phosphorus levels (P_2O_5 kg ha^{-1})				
30	3.36	0.896	0.398	0.198
40	3.56	0.955	0.425	0.210
50	3.64	0.956	0.435	0.214
SEm±	0.04	0.010	0.005	0.003
CD (*p* = 0.05)	0.10	0.029	0.014	0.009

Weed free treatment recorded the highest nitrogen uptake by seed, haulm as well as its total uptake (28.54, 20.53 and 49.08 kg ha^{-1}, respectively) and it was statistically at par with pendimethalin 750 g ha^{-1} as pre-emergence accompanied by imazethapyr + imazamox 33.75 g ha^{-1} as post-emergence (26.95 kg ha^{-1}) in terms of nitrogen uptake by seed only. Pendimethalin 1000 g ha^{-1}*fb* hoeing and weeding at 15-20 DAS, imazethapyr + imazamox 45 g ha^{-1}, pendimethalin 750 g ha^{-1} *fb* imazethapyr + imazamox 33.75 g ha^{-1}, one hoeing & weeding at 15-20 DAS and weed free up to 50 days tended to enhance nitrogen uptake by seed to an extent of 15.87, 13.58, 16.53, 12.59 and 18.12 kg ha^{-1}; by haulm 11.56, 9.80, 11.80, 9.40 and 13.09 kg ha^{-1} and total nitrogen uptake by the crop to the tune of 27.44, 23.38, 28.33, 21.99 and 31.22 kg ha^{-1}, respectively over weedy check. Similarly weed free treatment recorded the highest phosphorus uptake by seed, haulm as well as its total uptake (3.22, 4.34 and 7.57 kg ha^{-1}, respectively) which was statistically at par with pendimethalin 750 g ha^{-1} as pre-emergence in combination with imazethapyr + imazamox 33.75 g ha^{-1} as post-emergence (3.22, 4.34 and 7.57 kg ha^{-1}, respectively). The relative increase in phosphorus uptake by seed, haulm and total uptake owing to pendimethalin 1000 g ha^{-1}*fb* hoeing and weeding at 15-20 DAS, imazethapyr + imazamox 45 g ha^{-1}, pendimethalin 750 g ha^{-1}*fb* imazethapyr + imazamox 33.75 g ha^{-1}, one hoeing & weeding at 15-20 DAS and weed free up to 50 days was 1.91, 1.63, 1.99, 1.50 and 2.19 kg ha^{-1}; 2.53, 2.11, 2.67, 1.90 and 2.95 kg ha^{-1} and 4.44, 3.74, 4.67, 3.41 and 5.14 kg ha^{-1}, respectively over weedy check.

Application of phosphorus at 40 kg P_2O_5 ha^{-1} substantially influenced nitrogen and phosphorus uptake by seed, haulm and total uptake over 30 kg P_2O_5 ha^{-1} and further addition of phosphorus from 40 to 50 kg P_2O_5 ha^{-1} failed to enhance N uptake by seed, haulm and total uptake.

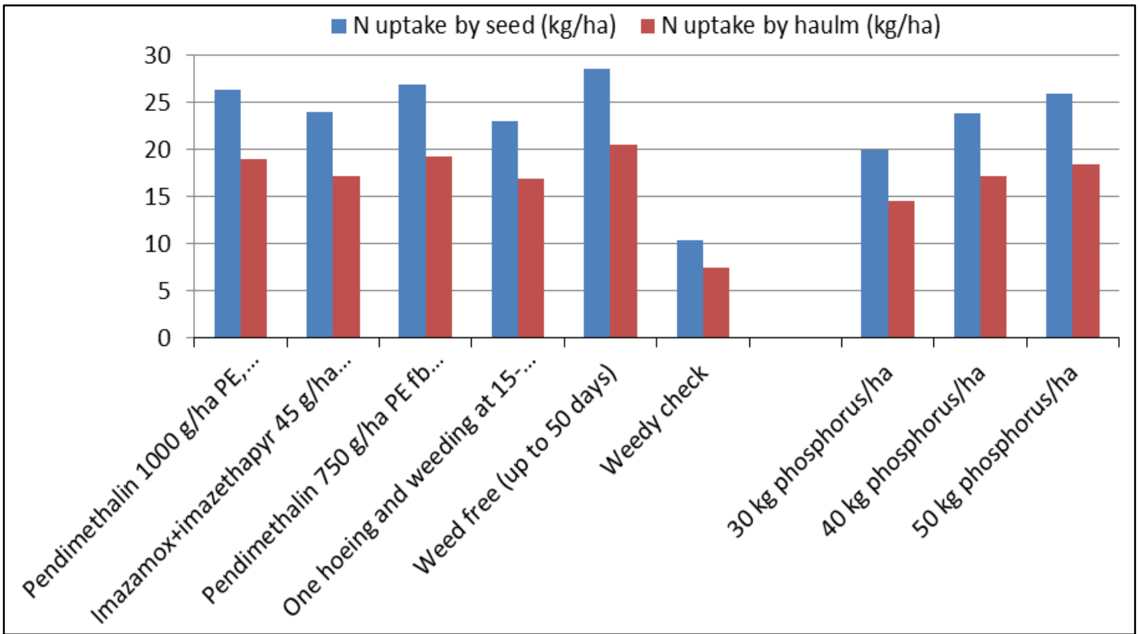

Fig 1: Effect of weed management and phosphorus levels on N uptake by seed and haulm of cowpea

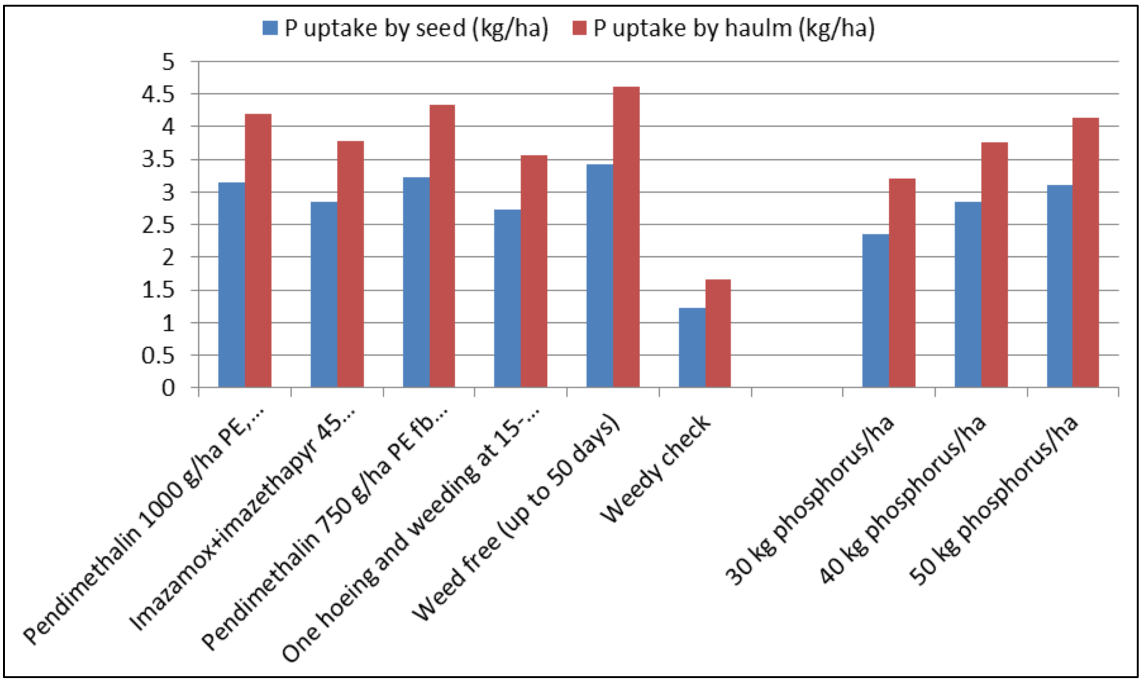

Fig 2: Effect of weed management and phosphorus levels on P uptake by seed and haulm of cowpea

Table 4: Effect of weed management and phosphorus levels on N and P uptake (kg ha^{-1}) by cowpea at harvest

Treatments	Nitrogen			Phosphorus		
	Seed	Haulm	Total	Seed	Haulm	Total
Weed management						
Pendimethalin 1000 g PE *fb* HW	26.29	19.00	45.30	3.14	4.20	7.34
Imazethapyr + imazamox 45 g	24.00	17.24	41.24	2.86	3.78	6.64
Pendimethalin 750g PE *fb* imazethapyr + imazamox 33.75 g	26.95	19.24	46.19	3.22	4.34	7.57
One hoeing and weeding 15-20 DAS	23.01	16.84	39.85	2.73	3.57	6.31
Weed free (up to 50 days)	28.54	20.53	49.08	3.42	4.62	8.04
Weedy check	10.42	7.44	17.86	1.23	1.67	2.90
SEm±	0.59	0.43	1.01	0.07	0.10	0.17
CD (p = 0.05)	1.68	1.22	2.88	0.20	0.29	0.48
Phosphorus levels (P_2O_5 kg ha^{-1})						
30	19.90	14.50	34.40	2.36	3.20	5.56
40	23.83	17.17	41.00	2.85	3.77	6.62
50	25.88	18.48	43.36	3.10	4.13	7.23
SEm±	0.83	0.60	1.43	0.10	0.15	0.24
CD (p = 0.05)	2.40	1.74	4.10	0.29	0.42	0.69

Conclusion
Based on one year field experimentation, it is obvious that maximum chlorophyll content, seed protein content, nitrogen and phosphorus content of seed and haulm and their uptake by seed, haulm and total uptake by cowpea crop were recorded with pre-emergence application of pendimethalin 750 g ha^{-1} in combination with imazethapyr + imazamox 33.75 g ha^{-1} as post-emergence at 15-20 DAS next to weed free treatment. On the other hand application of phosphorus at various doses influenced chlorophyll content, seed protein content, nitrogen and phosphorus content of seed and haulm and their uptake by seed, haulm and total uptake by cowpea crop significantly.

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