Original Research Article

Effect of preplant application of post emergent herbicide glyphosate on the events of germination, growth and biochemical composition of blackgram seedlings

ABSTRACT

Weed causes heavy yield loss when it competes with crops especially for light, water, nutrient and space. In crop production, weed management is a necessary operation to get a higher yield. Manual weeding operation is an easy and environmentally friendly method but it increases the cost of cultivation and makes the crop production practice a non-profitable business venture. Under this situation, the herbicide can be used as the most practical and effective alternative means of weed control. In intensive agriculture heavy use of herbicide affects the growth and biochemical composition of the crop. The objective of this study was to find out the effect of pre-plant soil application of post emergence glyphosate on the germination, growth and biochemical composition of blackgram seedlings. Results showed that glyphosate 1000 ppm did not significantly affect the germination of blackgram seeds. However, the higher concentration of 1000 ppm affects the root (1.08 cm) and shoot length (6.16 cm) compared to control (6.16 and 12.27 cm, respectively) and the lower concentration at the rate of 10 ppm enhance the root (6.26 cm) and shoot length (12.49 cm). Soluble protein content was higher in glyphosate 1000 ppm (70.60 mg g^{-1}**)** compare to control (59.40 mg g^{-1}**)**. The finding concludes that the pre-plant application of post emergence herbicide glyphosate didn't affect the germination. However it has reduced the root and shoot length of blackgram when applied at higher concentrations. Contrary to the higher dose the lower dose of glyphosate stimulates the growth and development of blackgram seedlings.

Keywords: Blackgram, Germination, Glyphosate, Soluble protein

1. INTRODUCTION

Blackgram (*Vigna mungo L.*) is one of the important legume crop grown throughout India. It contains about 24% of protein and is used as an important protein source in the human diet. The low productivity in blackgram is attributed to numerous biotic and abiotic factors, of which the most important factor is weed management. Generally pulse crops are cultivated in sequence after a cereal crop. Sowing of pulse crop in the sequence may get delayed due to excess moisture or lack of sufficient moisture. In the meantime, weeds will start to grow and occupy the entire field. In the presence of weeds the crops cannot be sown. Though the manual and mechanical are effective means to control weeds, these are time consuming and energy expensive. Hence the choice of chemical weed management is an intelligent and cost effective one. Among the herbicides recommended for weed management in the non-cropped fields, glyphosate is most suitable post emergence systemic herbicide to kill all the emerged weeds leaving no residue after application. However, famers and scientist are having opinion of the post emergence herbicides may have some phytotoxic effect on the subsequent crop sown immediately after harvest of previous crop. Hence a trail was laid out to find out the

effect of different dose of glyphosate on emerged weeds and its residual toxicity on the germination and growth events of blackgram crop.

Glyphosate is a non-selective, systemic, post-emergence herbicide, directly applied to plant foliage. In plants, glyphosate affects the shikimic acid pathway through inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase. Lack of EPSP production leads to a reduction in the aromatic amino acid synthesis that is vital for protein synthesis (Siehl, 1997). Glyphosate is absorbed through the leaves and stems of plants and is translocated throughout the plant. After the application of glyphosate, the plants produce the symptoms like stunted growth, loss of green colouration, leaf wrinkling or malformation and tissue death (Vencill, 2002).

Plants treated with glyphosate normally die within 1–3 weeks (Chang *et al*., 2002) and it degrades at a relatively rapid rate in most soils, with a half-life estimated between 7 and 60 days (Giesy *et al*., 2000). Glyphosate didn't apply as a pre-plant or pre-emergence herbicide to crops, it only applied as a post-emergence herbicide to weeds. The low dose of herbicide stimulates the growth and yield of the target plant, this growth-stimulating phenomenon of herbicides is known as herbicide hormesis (Calabrese *et al*., 2003). The low dose of glyphosate can have a positive effect on the early development of crop growth especially increasing plant height and biomass or increasing root and shoot length. Higher doses of glyphosate show its phytotoxic impact and kill plants but when glyphosate is applied to plants at 10 % of the rate recommended in field conditions, it promotes crop growth (Asman *et al*., 2003). In this study, detailed investigation was carried out to find out the effect of pre-plant application of glyphosate on the germination, morphological and biochemical changes in blackgram.

2. MATERIAL AND METHODS

The laboratory experiment was conducted in November 2021 at Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore. Four levels of glyphosate viz., 1000 ppm (T_1), 500 ppm (T_2), 100 ppm (T_3), 10 ppm (T_4) with one absolute control (T_5) were tested for its effects on the germination, growth and biochemical composition of germinating blackgram. In the laboratory study, the glyphosate was directly applied to blackgram seeds which were placed on a petri dish. Healthy blackgram seeds (VBN 8) were selected and soaked in distilled water for 30 min. Ten healthy seeds were placed on germination blotter paper in each petri dish and 4 ml of glyphosate at different concentrations (1000, 500, 100 and 10 ppm) was added to a respective petri dish. The different concentration of glyphosate was prepared using the active ingredient and distilled water was added to the absolute control treatment. Germination and seedling growth were recorded for up to 7 days at an interval of 24 hours. The experiment was conducted in a completely randomized design (CRD) with four replications. Soluble protein content was estimated from blackgram seedlings at 7 days after application of glyphosate by measuring the colour developed by the reduction of folin-ciocalteau reagent, following the method suggested by Lowry *et al*. (1951) and expressed in mg g^{-1} fresh weight of leaf. Proline content was estimated from blackgram seedlings at 7 days after application of glyphosate. Proline is an amino acid, protects the plants from various stress and helps the plants to recover from stress more rapidly. During the selective extraction with aqueous sulphosalicylic acid, proteins are precipitated as a complex. The extracted proline is made to react with ninhydrin in acid medium conditions to form the chromophore (red colour pigment complex) and read at 520 nm.

Observations on germination percentage, root length (cm), shoot length (cm), soluble protein content (mg g^{-1}) and proline content (mg g^{-1}) were recorded during the course of this experiment. The experimental data were statistically analysed by adopting Fischer's method of "Analysis of variance" as per Gomez and Gomez (1984). Wherever the treatment differences were found significant, the critical differences were worked out at a 5 percent probability level.

3. RESULTS AND DISCUSSION

3.1 Seed germination (%):

Different levels of glyphosate did not significantly affect the germination of blackgram seeds (Table 1). Similar finding was reported by Moshier Loren *et al*. (1976) in turfgrass. In our experiment, all the seeds used for this experiment were germinated at 1 day after application (DAA) of glyphosate. At 3 DAA the seeds in the control treatment were produced leaves. However, the blackgram seeds treated with glyphosate at the rate of 1000 ppm did not produce any leaves.

Glyphosate was applied at the rate of 0.56 and 1.12 kg ha^{-1} did not affect germination of treated grain sorghum (Bovey *et al*. 1975). Klingman *et al*., 1976 observed that application of glyphosate at 4.5 kg ha^{-1} produced no significant effect on germination percentages of the three grass species (*Poa pratensis* L., *Festuca rubra* L. and *F. arundinacea* schreb) when applied to the soil before seeding or when applied directly over the seeds.

3.2 Root and Shoot length (cm):

The highest root length of 6.26 cm was observed in glyphosate 10 ppm and it was on par with control which registered 6.16 cm of root length. The lowest root length (1.08) was noticed in glyphosate 1000 ppm and it was on par with glyphosate 500 ppm and glyphosate 100 ppm recorded 1.21 and 1.95 cm, respectively (Table 1) (Fig. 2).

The maximum shoot length (12.47 cm) was noted in glyphosate 10 ppm and it was on par with control. The lower shoot length of 6.16 cm was registered in glyphosate 1000 ppm) it was on par with glyphosate 500 ppm which registered a shoot length of 7.76 cm (Table 1) (Fig. 2).

Glyphosate applied during the germination stage, did not affect the germination process of the blackgram. However, the root and shoot length of the seedlings were affected with the different concentrations of the herbicide. Glyphosate treated seeds take more time for seedling emergence compared to control. Seedling growth was reduced with increased glyphosate concentration. Compared to higher concentration, the lower concentration of glyphosate (10 ppm) increased the root and shoot length of blackgram. Ferrari *et al.* (2021) reported that the grain yield and the number of spikelets per panicle of rice were increased with the application of 10 g a.e. ha^{-1} of glyphosate at the floral differentiation stage. Glyphosate application of 10-70 g a.e ha^{-1} was increased the number of panicles in rice. The number of spikelets per panicle, 100 grain weight and grain yield were reduced when glyphosate was applied between 70 and 100 a.e ha^{-1}.

Root and shoot length of peas *(Pisum sativum)* was decreased 14.7 and 17.6 % respectively, at 4 ppm of glyphosate application (Mondal *et al*., 2017). Sanjay (2006) reported that the hypocotyl length of *Hibiscus cannabinus L*. was reduced with increase the concentration of the glyphosate (30.33, 21.0, 9.33, 3.0 and 0 mm of hypocotyl length at 5000, 10000, 15000, 20000 and 25000 ppm respectively, whereas in control it was 40.33

mm). Similarly, the length of the radicle was 11.33, 7.0, 2.7, 1.33 and 0 mm at 5000, 10000, 15000, 20000 and 25000 ppm respectively.

3.3 Proline content (mg g^{-1}):

Proline content of the 10 days old blackgram seedling was statistically non-significant. There is a positive correlation between the proline accumulation and plant stress. If the plants are under stressed condition, it accumulates more proline content compared to the normal plant. Glyphosate treated willow (*Salix miyabeana*) plant produce more proline content compared to control (Gomes *et al*.2017). Fayez *et al*., 1996 observed that the increase in proline content was highest in *Pisum* and *Vicia* after chlorsulfuron treatment, whereas triallate and norflurazon were less effective. An increase in proline was solely responsible for any of the ultrastructural abnormalities claimed to be due to the herbicides. It is more probable that the proline increases reflected stress due to the herbicide action.

3.4 Soluble protein (mg g^{-1}):

The highest soluble protein content (96.53 mg g^{-1}) was observed in glyphosate applied at the rate of 500 ppm and the lowest soluble protein content of 59.40 mg g^{-1} was recorded in control and it was on par with glyphosate 10 ppm (Table 2).

Maximum soluble protein content was observed with the stunted seedlings and less soluble protein was recorded with the developed seedlings. During seedling development, the storage protein is hydrolysed to free amino acids, these provide energy for seedling development. So, the fully developed seedlings contain less protein compared to undeveloped seedlings. This finding concludes that glyphosate didn't affect the soluble protein synthesis in blackgram.

Soluble protein was utilized for a large proportion (39.1–93.9%) from imbibition (stage 1) to the highest germination (stage 4) and even the early seedling stage. The protein is degraded by the proteases and converted to polypeptide and amino acids during germination (Palmiano and Juliano, 1972). These hydrolysis products may meet the energy needs of seeds for germination (Bewley and Black, 1994; Wahid and Bounoua, 2013). When seeds were started to germinate, the protein was broken down by the proteases to convert insoluble storage protein into soluble peptides, which are further hydrolysed to free amino acids. These free amino acids are mobilized to the embryonic axis to support its growth and also to provide energy (Shutov and Vaintraub, 1987).

4. CONCLUSION

Glyphosate is used as a post-emergence herbicide during the fallow period to kill the weeds grown and facilitate the tillage operation for the succeeding crop. Based on the experiments conducted it may be concluded that the application of glyphosate directly at lower concentration did not affect either germination process or growth of the blackgram seedlings. Increased concentration of glyphosate above 10 ppm, affects the root and shoot development and produces malformed seedlings. Glyphosate at the rate of 10 ppm increases the root and shoot length due to the hormesis effect. Soluble protein synthesis was normal and the proline was increased due to chemical stress. In conclusion, the glyphosate residue presents at low concentration in the soil did not affect germination and seedling development rather increased the root and shoot development of the young blackgram seedlings.

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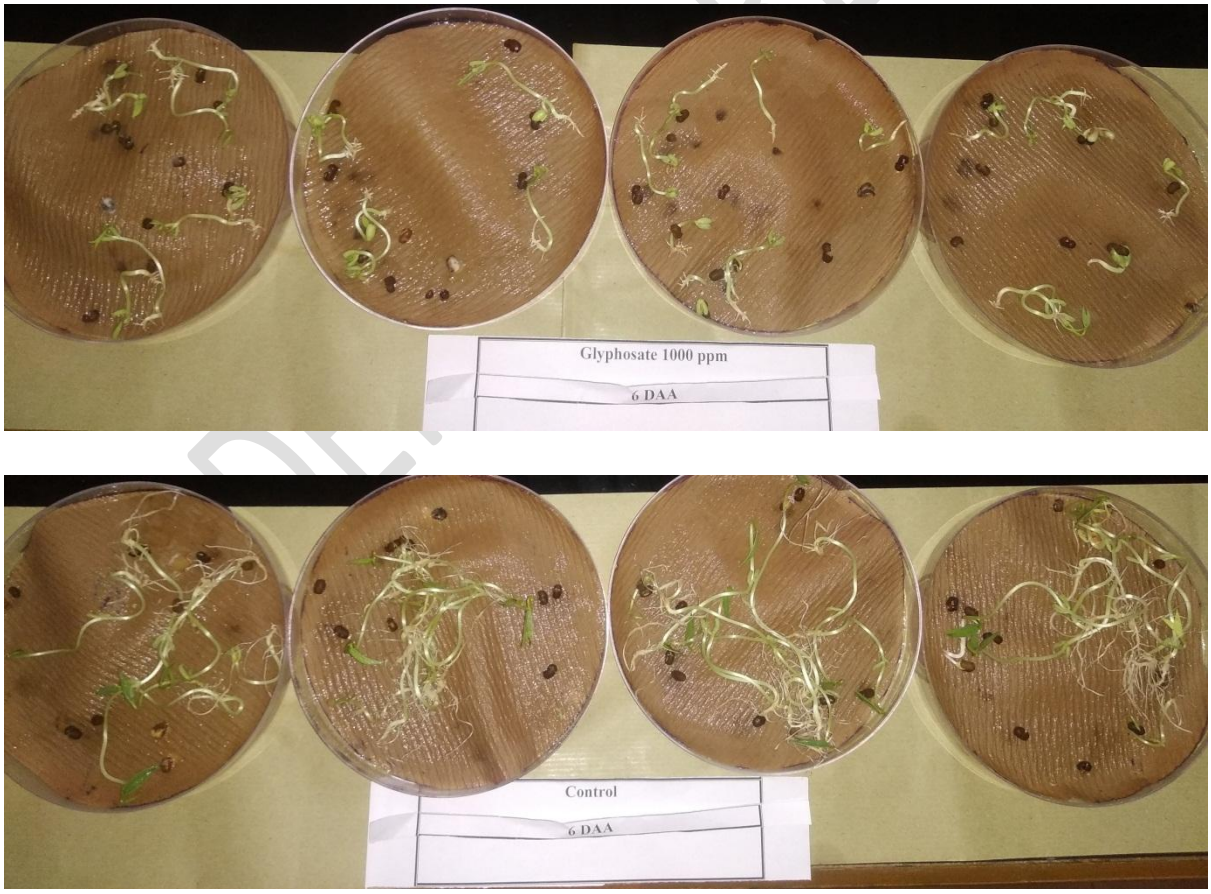

Fig. 1. Effect of glyphosate 1000 ppm and control on blackgram seedlings at 6 Days After Application (DAA)

Fig. 2. Effect of glyphosate on blackgram seedling growth at 10 Days After Application (DAA)

Table 1. Glyphosate effects on blackgram germination, root length and shoot length

Treatments	Germination (%)	Root length (cm)	Shoot length (cm)
T_1- Glyphosate 1000 ppm	97.5	1.08	6.16
T_2- Glyphosate 500 ppm	97.5	1.21	7.76
T_3- Glyphosate 100 ppm	100	1.95	8.58
T_4- Glyphosate 10 ppm	100	6.26	12.49
T_5- Control	100	6.16	12.27
SEd	**2.33**	**0.47**	**0.80**
CD (*P*=0.05)	**NS**	**0.99**	**1.70**

Table 2. Glyphosate effects on proline and soluble protein content in blackgram

Treatments	Soluble protein (mg g^{-1})	Proline content (mg g^{-1})
T_1- Glyphosate 1000 ppm	70.60	2.67
T_2- Glyphosate 500 ppm	96.53	0.96
T_3- Glyphosate 100 ppm	84.27	1.00
T_4- Glyphosate 10 ppm	61.67	1.11
T_5- Control	59.40	0.49
SEd	**2.38**	**0.87**
CD (*P*=0.05)	**5.30**	**NS**