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ORIGINAL PAPER

Biochemical Response of Plant and Soil to Varied Levels of Nitrogen and Penoxsulam Application in Rice Crop

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Abstract

Nutrient management and chemical weed control are the two important aspects in crop production. The interaction between nitrogen (N) fertilizer application and penoxsulam on the biochemical properties of rice plants and soil properties is still to be explored under sub-tropical conditions. Rice was grown for two seasons (2020 and 2021) with three levels of N (100, 125, and 150% of recommended dose) and three levels of penoxsulam 2.67% oil dispersion (OD) (0, 1000, and 2000 ml ha^{-1}). Plant and soil samples were collected at different growth stages of the crop. Plant samples were analyzed for chlorophyll a, chlorophyll b, total phenolic activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, and total nutrients. Soils from the respective plots were also analyzed for available nutrients and soil enzyme activities. Increased application of nitrogen improved chlorophyll pigments in rice, and the effect of penoxsulam on these pigments was negatively correlated. In contrary, penoxsulam increased the total phenolic content of rice leaf. The interaction of N and penoxsulam indicated an enhanced proline level in rice. Available nutrients in rice soils were increased with increasing application of N. The effect of N fertilization on soil enzymatic activities was positive, but declines with the application of penoxsulam. In rice grains, amylose content was decreased with N levels and increased with penoxsulam concentration. The highest rice yield was obtained with 125% N coupled with double the recommended dose of penoxsulam. Application of nitrogen (125%) coupled with 1000 ml ha^{-1} penoxsulam alters biochemical responses in plant and soil, which paved acceptable yield and grain quality.

Keywords Amylose · DPPH · Nutrients · Proline · Soil enzymes

1 Introduction

Rice (*Oryza sativa* L.) is the world's most important staple food crop. The crop is grown on a huge scale all throughout the world, and it ranks third in overall production behind sugarcane and maize. World rice production in 2022–2023 is now pegged at 516 million tonnes (FAO 2023), and its consumption in India is 109 million metric tonnes, second after China. Rice production might vary depending on a number of factors (biotic and abiotic). Among these, nutrient management and weed control are undoubtedly the two most important aspects. Nitrogen (N) is an essential element for plant growth and development. It is the building block element in a variety of organic molecules, including chlorophylls, cytochromes, enzymes, and coenzymes (Campestrini et al. 2014). It engages in photosynthetic processes (Nasar et al. 2022) as well as dry matter synthesis and accumulation (Feng et al. 2009). On the other hand, weed infestation may cause a reduction in the yield by 80–90% in irrigated rice crop (Langaro et al. 2018) which is usually managed through the application of chemical herbicides. These toxic substances, often known as weed killers, are biologically active agents that are used to suppress weeds while leaving the desired crop uninjured. Nitrogen application, which is crucial for plant growth and development, is also having a significant impact on crop-weed interactions. Furthermore, nitrogen quickly recovers plant harm caused by the toxic effects of various herbicides used (Nivelle et al. 2018).

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Although Soil Test Crop Response (STCR)-based nitrogen recommendation for rice is already established in different agro-climatic zones of India, farmers generously apply higher doses of nitrogenous fertilizers with the intention to maximize yields. Various price and non-price factors are also responsible for the increased use of nitrogen by the small and marginal farmers in the country (Pani et al. 2021). Alongside, due to a lack of technical knowledge and poor communication, it has been observed in many cases that farmers use herbicides in crops at higher doses. The injudicious use of nitrogen poses severe constraints on plant's biochemical characteristics (Elhanafi et al. 2019). Simultaneously, the use of selective herbicide in excess amount may affect photosynthetic activity and cause oxidative damage to the crop. It also triggers plants' self-defense mechanisms through various processes. At the same time, an unintended consequence of herbicide use could be disruption of microbial metabolism or enzymatic activities in soil microorganisms (Bowles et al. 2014; Bhatt et al. 2018). Among the soil biochemical properties, injudicious application of nitrogen fertilizers may alter enzyme activity in rice rhizosphere which could have a considerable impact on soil health *vis-à-vis* plant productivity (Rakshit et al. 2015). This led the researchers to study the impact of excess nitrogen and herbicides on biochemical changes in rice plants and soil independently. Although several literatures are available on the biochemical alteration of rice plant and rhizosphere due to the application of excess nitrogen and herbicides, there exists a gap on understanding the unexplored variations in response to the interaction effect of these two factors.

Penoxsulam {2-(2,2-difluoroethoxy)-N-5,8-dimethoxy[1,2,4]triazolo[1,5-C]pyrimidin-2-yl-6-(trifluoromethyl) benzene-sulfonamide}, a member of the triazopyrimidine sulfonamide chemical family, is a novel post-emergence, broad-spectrum rice herbicide, recently registered in India with two different formulations such as 21.7% SC and 2.67% OD (CIBRC 2022). The compound is a systemic herbicide that is absorbed primarily via the leaves and secondarily through the roots. The behavior of this new herbicide in subtropical climate is still not known clearly. Existing ambiguity regarding the interaction effect between excess nitrogen and herbicide (penoxsulam) on quantitative as well as qualitative attributes of rice and soil needs to be explored. Considering penoxsulam as a newly introduced herbicide in India, in this study, we hypothesized that the interaction of excess N fertilization and penoxsulam could have considerable implications on the biochemical properties of rice, its yield, and associated changes in the soil enzyme activities. To test this hypothesis, a field experiment was conducted to generate meaningful information regarding the effects of nitrogen and penoxulam herbicide on various biochemical behavior of the rice ecosystem at different growth stages of the crop and to ascertain the changes in enzymatic activity of soil.

2 Materials and Methods

2.1 Experimental Details

Transplanted paddy (var. Sabour Dweep; spacing 20 × 15 cm) was grown in the rainy season (July 2020 and 2021) at Bihar Agricultural College farm, Sabour (25°50′N latitude, 87°19′E longitude, and 37.19 m altitude) in Factorial Randomized Block Design (FRBD) with three factors (growth stages, doses of penoxsulam, and doses of nitrogen) with three replicates.

2.2 Treatments

Penoxsulam (Fig. S1) 2.67% OD was applied in three levels, i.e., 1000 ml ha^{-1} (H_{1000}: recommended dose; a.i.-26.7 g), 2000 ml ha^{-1} (H_{2000}: double the recommended dose; a.i.-53.4 g) at 25 days after transplanting (DAT) along with an untreated control plots (H_0: no herbicide). Three levels of nitrogenous fertilizer: 100% (N_{100}), 125% (N_{125}), and 150% (N_{150}) recommended dose of N (i.e., 120 kg ha^{-1}) were applied at three equal splits: on the day of transplanting followed by two top dressings (25 and 50 DAT). Phosphorous (P_2O_5: 60 kg ha^{-1}) and potassium fertilizer (K_2O: 40 kg ha^{-1}) were applied as per the recommendation for the rice variety.

2.3 Plant and Soil Analyses

Plant leaf samples were collected from each plot at early tillering (S_1: 24 DAT), mid-tillering (S_2: 35 DAT), Panicle Initiation (PI) (S_3: 49 DAT), and anthesis (S_4: 70 DAT). Chlorophyll content (a and b) in green leaves was measured in an ultraviolet–visible (UV–VIS) spectrophotometer at 645 and 663 nm wavelengths using dimethyl sulfoxide (Barnes et al. 1992). Total phenolic content was estimated colorimetrically at 750 nm using gallic acid as a standard reagent (Singleton and Rossi 1965). DPPH (2, 2'-diphenyl-1-picrylhydrazyl) scavenging activity was determined in leaf samples to evaluate the presence of natural antioxidants in rice plants (Beniwal and Jood 2014). The samples were homogenized with ethanol, and then, DPPH was added and finally estimated at 517 nm in the UV–VIS spectrophotometer. Proline, an amino acid, is generally accumulated in high amounts in plants when exposed to stress. It is being determined in the experiment by extracting leaf samples with sulfosalicylic acid, followed by centrifugation, and finally measuring the absorbance of the aliquot at 520 nm (Bates et al. 1973). Grains from the respective plots were harvested during the maturity stage and processed to determine the amylose content by following the spectrophotometric method using starch-iodine solution (Avaro et al. 2011). Important nutrients like total N (Bremner and Mulvaney 1982), P (Piper 1967), K (Koenig and Johnson 1942), Fe, and Zn (Elwell and Gridley 1967) content along with total phenolic content and DPPH scavenging activity in grains were also being measured as per standard methodology.

Soil samples were collected from 15 cm depth of each plot at early tillering (S_1: 24 DAT), PI (S_2: 49 DAT), anthesis (S_3: 70 DAT), and at harvest (S_4). Soil samples were analyzed for pH (Jackson 1973), EC (Jackson 1973), organic carbon (Walkley and Black 1934), available N (Subbiah and Asija 1956), P (Watanabe and Olsen 1965), K (Schollenberger and Simon 1945), Fe, and Zn (Lindsay and Norvell 1978). Enzyme activities were determined by measuring the end product after soils were incubated with specific substrates. Dehydrogenase activity was measured using 3% triphenyl tetrazolium chloride (TTC) as a substrate, and the intensity of Triphenyl Formazan (TPF) formed was estimated by taking the absorbance in a spectrophotometer at a wavelength of 485 nm (Klein et al. 1971). P-nitrophenyl phosphate solution was used as a substrate for acid (ACP) and alkaline phosphatase activity (AKP) buffered at 6.0 and 11.0, respectively, using modified universal buffer (MUB), and the intensity of the yellow color of the final product (p-nitrophenol) was assessed at 440 nm in a spectrophotometer (Tabatabai and Bremner 1969). For fluorescein diacetate (FDA) hydrolysis activity, the final product fluorescein was assessed by taking the absorbance using a spectrophotometer set to a 490 nm wavelength (Green et al. 2006).

2.4 Statistical Analysis

ANOVA pertaining to the levels of nitrogen and penoxsulam with respect to different growth stages of rice was calculated following standard statistical methods (Gomez and Gomez 1984). Duncan's multiple range test (DMRT) for comparison of means was performed using SAS (version 9.2; SAS Institute, Cary, North Carolina, USA), and graphs were prepared with SIGMAPLOT (version 14.0; Systat Software, San Jose, California, USA). Unless otherwise stated, the level of significance referred to in the results is $P < 0.05$.

3 Results

3.1 Analysis of Plant Samples

3.1.1 Chlorophyll Content

Application of nitrogen and penoxsulam has a significant effect on the production of both the plant pigments, namely, chlorophyll a and chlorophyll b, in all the growth stages of rice except the early tillering stage after which penoxsulam application was applied. Irrespective of the growth stages, both the pigments were found highest at 150% nitrogen level without application of herbicide (Fig. 1). The herbicide caused a significant decrease in both the pigment's level at mid-tillering, PI, and anthesis stage (Table 1). Analysis of interactions among nitrogen and penoxsulam did not reveal any significant effects on chlorophyll content in the early tillering stage; however, these interactions were significant in the later stages of the crop (Table 5).

3.1.2 Total Phenolic Content

Penoxsulam significantly increased the total phenolic content of rice plants at the subsequent growth stages of rice (Table 1). Increasing nitrogen level did not significantly change the corresponding values for rice. Interaction between nitrogen and penoxsulam was non-significant ($P > 0.05$ in all the stages of crop growth).

3.1.3 DPPH Scavenging Activity

Change in DPPH scavenging activity also followed the same trend as observed with total phenolic content (Table 1).

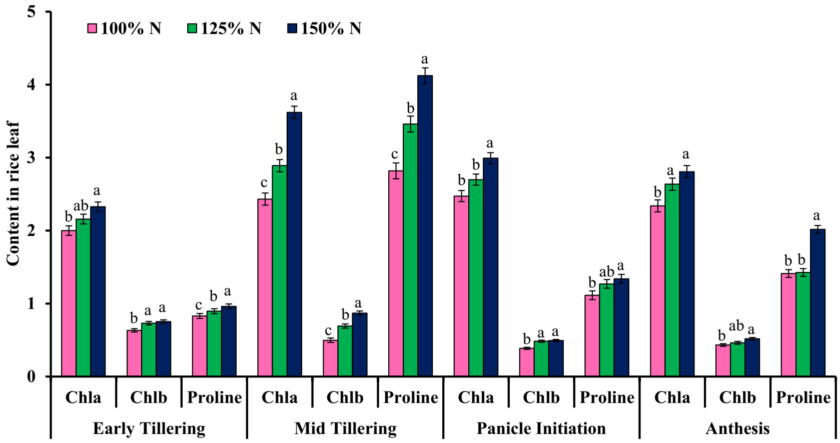

Fig. 1 Effects of nitrogen levels on chlorophyll a (Chla), chlorophyll b (Chlb) (mg chlorophyll g^{-1} fresh weight of leaf), and proline content (μg proline g^{-1} fresh weight of leaf) in rice leaf at different growth stages of rice. Bars with different letters are significant at $P < 0.05$ (Duncan's multiple range test), when respective parameter is compared separately over different growth stages. Error bars represent the standard error of the means

Table 1 Amount of chlorophyll a, chlorophyll b (mg chlorophyll g^{-1} fresh weight of leaf), total phenolic content (mg gallic acid equivalent g^{-1} fresh weight) of sample, and DPPH scavenging activity (%) in rice leaf influenced by different levels of penoxsulam at different growth stages of rice. Mean data marked with different letters are significant at $P < 0.05$ (Duncan's multiple range test), when respective parameter is compared separately over different growth stages

Treatments/ stage	Mid-tillering				Panicle initiation				Anthesis			
	Chlorophyll a	Chlorophyll b	Total phenolic content	DPPH scavenging activity	Chlorophyll a	Chlorophyll b	Total phenolic content	DPPH scavenging activity	Chlorophyll a	Chlorophyll b	Total phenolic content	DPPH scavenging activity
No herbicide	3.51a	1.40a	0.26c	21.05c	3.27a	0.69a	0.23c	18.50c	3.19a	0.68a	0.17c	15.86c
RD	2.95b	0.44b	0.57b	44.97b	2.60b	0.45b	0.27b	35.38b	2.58b	0.52b	0.21b	19.01b
DRD	2.48c	0.22c	0.82a	62.31a	2.29c	0.22c	0.32a	44.31a	2.01c	0.21c	0.24a	22.37a
SEM	0.08	0.03	0.02	1.37	0.08	0.01	0.01	0.99	0.08	0.02	0.01	0.60

SEM represents the standard error of the means

RD, recommended dose-H_{1000} (1000 ml ha^{-1})

DRD, double the recommended dose-H_{2000} (2000 ml ha^{-1})

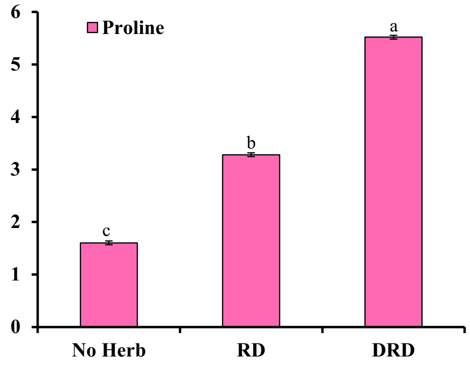

Fig. 2 Effects of penoxsulam levels [RD—recommended dose (H_{1000}-1000 ml ha^{-1})] and [DRD—double the recommended dose (H_{2000}-2000 ml ha^{-1})] on proline content (μg proline g^{-1} fresh weight of leaf) in rice leaf at the mid-tillering stage. Bars with different letters are significant at $P < 0.05$ (Duncan's multiple range test). Error bars represent the standard error of the means

Herbicide application significantly increased the activity in rice plant in subsequent growth stages. Interaction between nitrogen and penoxsulam followed the same trend as that of total phenolic content.

3.1.4 Proline Content

An increase in nitrogen level had consequently enhanced proline level in rice in all the growth stages (Fig. 1). At the mid-tillering stage, penoxsulam application was found to increase proline level in plant (Fig. 2), and interaction between nitrogen and the herbicide had a significant effect on the same at that particular stage (Table 5).

3.2 Analysis of Soil Samples

3.2.1 Soil pH, Electrical Conductivity (EC), Organic Carbon (OC)

No significant change was observed in soil pH, EC, and OC content due to change in both nitrogen and the herbicide level (data not presented).

3.2.2 Available Nutrients

Soil available N, P, and K had been significantly increased with the increase in nitrogen fertilizer level throughout the growth phases of rice (Fig. 3). Penoxsulam did not have any significant effect on the availability of those nutrients in the soil. No significant change in DTPA zinc and iron level had been observed due to change in nitrogen as well as penoxsulam level (data not presented). We could not locate any interaction effect of nitrogen and penoxsulam on soil parameters (Table 6).

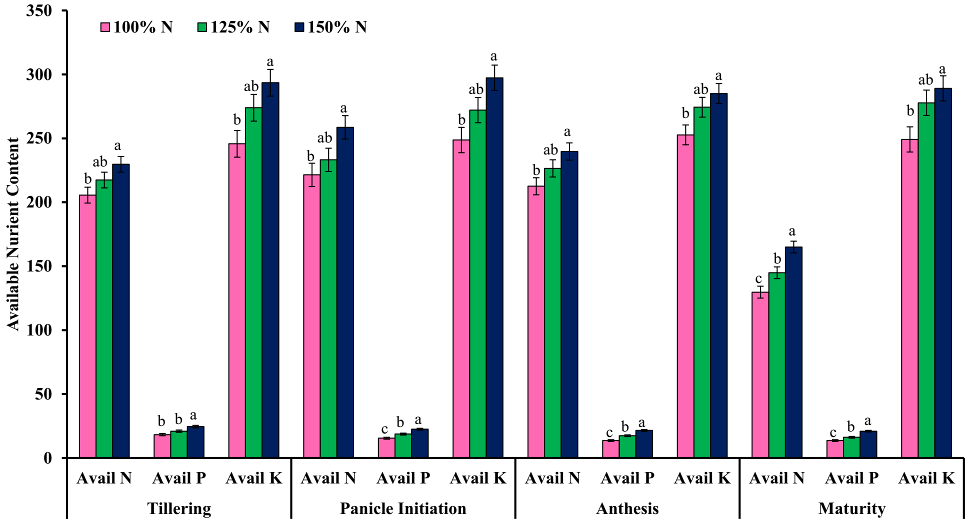

Fig. 3 Effects of nitrogen levels on available nitrogen (avail N), available phosphorous (avail P), and available potassium (avail K) (kg ha^{-1}) in soil at different growth stages. Bars with different letters are significant at $P < 0.05$ (Duncan's multiple range test), when respective parameter is compared separately over different growth stages. Error bars represent the standard error of the means

3.2.3 Soil Enzymes

An increase in nitrogen level caused a significant increase in all the four soil enzymatic activities studied at tillering, PI, and anthesis stages of rice (Fig. 4). These activities were found maximum at the anthesis stage. However, penoxsulam significantly decreased these phenomena at the PI stage of rice (Fig. 5). Similar to soil parameters, there was no significant interaction effect observed on the soil enzymes (Table 6).

3.3 Analysis of Grain Samples at Harvest

3.3.1 Total Phenolic Content

A significant increase in the total phenolic content of rice grains had been observed due to an increase in penoxsulam level (Table 2). However, increasing nitrogen level did not significantly change the corresponding values for grains. No interaction effect was observed in the total phenolic content of grains (Table 3).

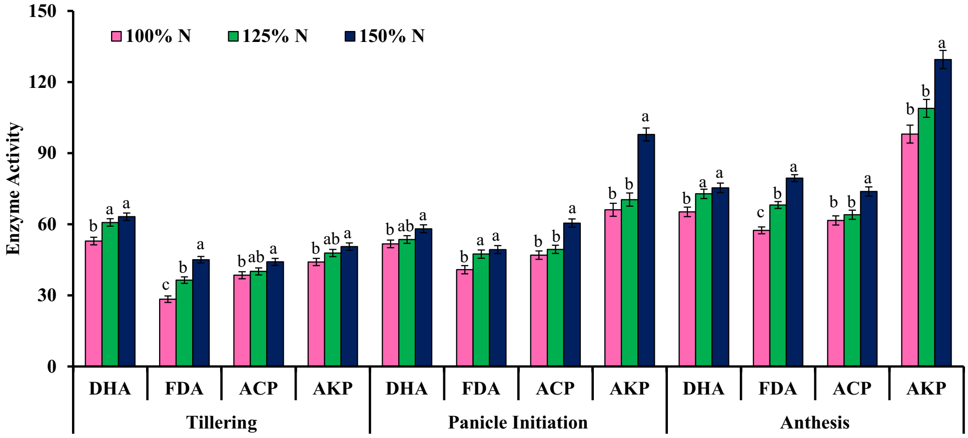

Fig. 4 Effects of nitrogen levels on dehydrogenase activity (DHA-µg TPF released g^{-1} dry soil h^{-1}), fluorescein diacetate hydrolyzing capacity (FDA—µg fluorescein g^{-1} dry soil h^{-1}), acid phosphatase activity (ACP—µg p-nitrophenol released g^{-1} dry soil h^{-1}), and alkaline phosphatase activity (AKP—µg p-nitrophenol released g^{-1} dry soil h^{-1}) in soil at different growth stages. Bars with different letters are significant at $P < 0.05$ (Duncan's multiple range test), when respective parameter is compared separately over different growth stages. Error bars represent the standard error of the means

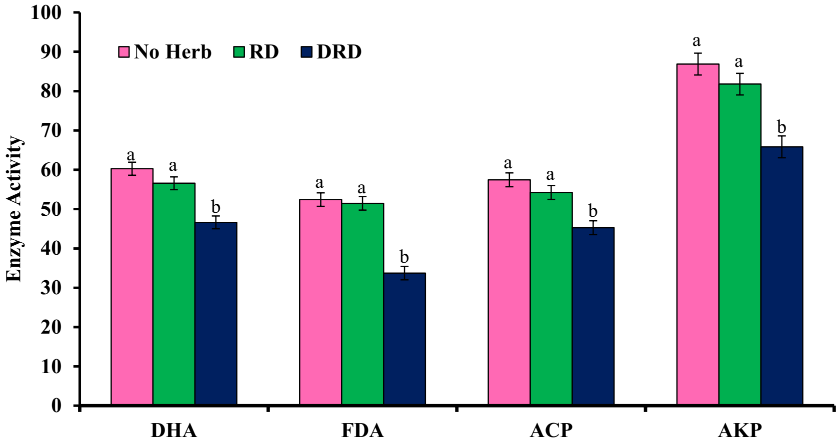

Fig. 5 Effects of penoxsulam levels [RD—recommended dose (H_{1000}-1000 ml ha^{-1})] and [DRD—double the recommended dose (H_{2000}-2000 ml ha^{-1})] on dehydrogenase activity (DHA—μg TPF released g^{-1} dry soil h^{-1}), fluorescein diacetate hydrolyzing capacity (FDA—μg fluorescein g^{-1} dry soil h^{-1}), acid phosphatase activity (ACP—μg p-nitrophenol released g^{-1} dry soil h^{-1}), and alkaline phosphatase activity (AKP—μg p-nitrophenol released g^{-1} dry soil h^{-1}) in soil at the panicle initiation stage. Bars with different letters are significant at $P < 0.05$ (Duncan's multiple range test). Error bars represent the standard error of the means

Table 2 Amounts of total phenolic content (mg gallic acid equivalent g^{-1} fresh weight), DPPH scavenging activity (%), and amylose content (%) in rice grain influenced by different levels of penoxsulam at harvest. Mean data marked with different letters within each column are significant at $P < 0.05$ (Duncan's multiple range test)

Treatments	Total phenolic content	DPPH scavenging activity	Amylose content
No herbicide	0.87b	67.31b	17.98b
RD	1.05a	79.90a	19.67ab
DRD	1.10a	81.97a	20.24a
SEM	0.03	2.06	0.58

SEM represents the standard error of the means

RD, recommended dose-H_{1000} (1000 ml ha^{-1})

DRD, double the recommended dose-H_{2000} (2000 ml ha^{-1})

3.3.2 DPPH Scavenging Activity

DPPH scavenging activity got increased due to an increase in penoxsulam level (Table 2). Raise in nitrogen level did not change the activity significantly (Table 3).

3.3.3 Amylose Content

The amylose content of rice grains had been decreased significantly with the increase level of nitrogen fertilizer. However, application of the herbicide caused a significant increase of amylose in grains (Table 2). The interaction between nitrogen and penoxsulam was non-significant (Table 3).

3.3.4 Nutrient Content

Both the application of nitrogen and herbicide at different doses did not cause any significant change in the total N, P, K, Zn, and Fe content of grains (data not presented).

Table 3 Relative changes in total phenolic content (mg gallic acid equivalent g^{-1} fresh weight), DPPH scavenging activity (%), and amylose content (%) in rice grain due to application of nitrogen and penoxsulam

Treatments	Total phenolic content			DPPH scavenging activity			Amylose content		
	C_1	C_2	P	C_1	C_2	P	C_1	C_2	P
N_{100}	1.23	2.68	0.807	0.04	1.42	0.915	− 1.03	− 14.44	**0.003**
H_0	21.44	27.34	**0.000**	18.70	21.79	**0.000**	10.06	13.23	**0.021**
NXH	-	-	0.940	-	-	0.990	-	-	0.224

For nitrogen (N): C_1 and C_2 represent relative changes against N_{125} (125% of recommended N) and N_{150} (150% of recommended N), respectively

For herbicide (H): C_1 and C_2 represent relative changes against H_{1000} (1000 ml ha^{-1}) and H_{2000} (2000 ml ha^{-1}), respectively

The bold values are significant at $P < 0.05$

3.3.5 Grain Yield

Both the levels of herbicide and nitrogen individually and their interaction had a significant effect on yield. The highest yield was obtained in the plot using double the recommended dose of herbicide and 125% nitrogen (Table 4).

4 Discussion

The present study has dealt with important aspect of penoxsulam and nitrogen application in paddy and their interaction at different growth stages of the crop. The herbicide, penoxsulam, is a new input in Indian agriculture. As a result, using this novel chemical may possibly cause changes in the soil–plant continuum. Hence, this study was designed with rice crop grown in two seasons; however, we could not acquire a significant effect of season on the plant and soil parameters. Only a few significant interactions were observed especially in chlorophyll and proline contents in rice leaves (Tables 5 and 6).

Penoxsulam may impede chlorophyll (a and b) development quickly after application in the field, according to the findings of the study. This observation is supported by Netherland et al. (2009) who reported that penoxsulam inhibited chlorophyll "a" formation in algae. Linu and Girija (2020) reported that higher doses of penoxsulam reduced the chlorophyll (a and b) content in rice. This could be because herbicides in general hinder the common enzyme that connects the chlorophyll and cytochrome synthesis pathways, resulting in the creation of an intermediate tetrapyrrole that prevents chlorophyll pigment formation (Matringe et al. 1989). Nitrogen, on the other hand, had the opposite effect, boosting the green pigmentation of the crop. In the current experiment, nitrogenous fertilizer had significantly increased chlorophyll (a and b) formation. After 1st top dressing at 25 DAT, rice plants started showing visual symptoms of increasing greenness. At the mid-tillering stage, chlorophyll contents were found highest compared to other growth stages. A positive correlation between nitrogen doses and the amount of chlorophyll formed in the plant in all the combinations is well documented in several literatures (Hou et al. 2020; Peng et al. 2021). Phenolic compounds are significant plant constituents responsible for antioxidant activity. Studies on the effects of penoxsulam on the phenolic contents of rice are very scarce. In the present experiment, the total phenolic content of rice got increased soon after the application of herbicide, i.e., at the mid-tillering stage. Thereafter, it started to decline and found less in subsequent growth stages. Higher doses of penoxsulam caused increased production of this antioxidant composition in rice. Zarzecka et al. (2019) found that the application of herbicide in potato field raised polyphenol contents as a defense mechanism showed by the plant under stress. Total phenolic content was found highest in grains at maturity. A similar observation was found by Chen and Bergman (2005) in another experiment. Numerous scientific studies have been planned to look into the effect of nitrogen fertilizer on the concentration of phenolic compounds in plants and their antioxidant capability. The reported findings, however, were inconclusive. There are studies that reveal both positive and negative effects of nitrogen fertilizer on the concentration of phenolic compounds in plants (Amarowicz et al. 2020). The present study did not find any significant effect of nitrogen on the phenolic content of rice. Simultaneously, it was observed that there was a positive correlation between phenolic content and DPPH scavenging activity irrespective of the growth stages, levels of nitrogen, and penoxsulam. The finding is supported by Rayee et al. (2020) who found that an increase in radical scavenging activity in rice could be linked to an increase in total phenolic content. Beniwal and Jood 2014 also reported that the phenolic content of Bengal gram seed coat extract was substantially higher, as was the antioxidant activity. Proline is reportedly being found in plants to play a crucial role in coping up several stresses (Nguyen et al. 2021). Excessive accumulation of proline induces plant stress tolerance by maintaining optimal concentration of cell electrolytes, resisting membrane leakage, and maintaining redox potential (Hayat et al. 2012). In this experiment, a higher dose of nitrogen stress caused an increased level of proline which is supported by the findings of Wang et al. (2012). The herbicide, after application at 25 DAT, might have also raised stress levels at the mid-tillering stage, which may be alleviated due to increased proline production by rice.

Application of nitrogen at different doses increased the availability of N, P, and K significantly. The availability of N was increased because the nitrogen fertilizer was applied at an increasing rate and as split doses which was well in accordance with the finding of Tu et al. (2014). The result

Table 4 Rice grain yield (t ha^{-1}) as influenced by varied levels of nitrogen and penoxsulam. Mean data marked with different letters within each column are significant at $P < 0.05$ (Duncan's multiple range test)

Treatments	100% N	125% N	150% N	Mean
No herbicide	2.98d	3.94bc	3.76c	3.56
RD	4.00abc	4.39a	4.05abc	4.14
DRD	4.24ab	4.41a	4.16abc	4.27
Mean	3.74	4.25	3.99	
SEM		0.14 (NXH)		

SEM represents the standard error of the means

RD, recommended dose-H_{1000} (1000 ml ha^{-1})

DRD, double the recommended dose-H_{2000} (2000 ml ha^{-1})

Table 5 Relative changes (%) in chlorophyll a, chlorophyll b, total phenolic content, DPPH scavenging activity, and proline content in leaf due to application of nitrogen and penoxsulam at different growth stages of rice

Treatments/stage	Chlorophyll a			Chlorophyll b			Total phenolic content			DPPH scavenging activity			Proline		
	C_1	C_2	P	C_1	C_2	P	C_1	C_2	P	C_1	C_2	P	C_1	C_2	P
Early tillering															
N_{100}	7.89	16.30	**0.011**	15.53	19.02	**0.006**	0.47	2.33	0.921	−0.03	3.79	0.565	7.90	15.93	**0.045**
H_0	−0.16	2.42	0.809	−0.83	−2.20	0.879	0.45	-7.21	0.363	−0.47	6.83	0.165	−0.50	−0.12	0.995
NXH	-	-	0.394	-	-	0.117	-	-	0.185	-	-	0.124	-	-	0.995
Mid-tillering															
N_{100}	18.86	48.90	**0.000**	39.26	74.79	**0.000**	2.73	8.82	0.231	1.42	2.08	0.899	22.74	46.28	**0.000**
H_0	−15.95	−29.54	**0.000**	−68.39	−84.12	**0.000**	117.52	216.24	**0.000**	113.64	196.04	**0.000**	104.72	244.52	**0.000**
NXH	-	-	**0.041**	-	-	**0.000**	-	-	0.585	-	-	0.872	-	-	**0.032**
Panicle initiation															
N_{100}	9.11	20.96	**0.001**	25.18	27.41	**0.000**	−0.41	−0.41	0.998	8.85	11.82	0.052	13.96	20.24	**0.048**
H_0	−20.37	−29.78	**0.000**	−34.36	−68.40	**0.000**	18.36	37.20	**0.001**	91.23	139.51	**0.000**	1.53	0.18	0.971
NXH	-	-	**0.047**	-	-	**0.006**	-	-	0.681	-	-	0.783	-	-	0.994
Anthesis															
N_{100}	12.68	20.03	**0.004**	7.03	19.68	**0.016**	2.76	6.63	0.381	3.32	5.39	0.512	1.10	42.91	**0.000**
H_0	−19.21	−36.89	**0.000**	−22.95	−68.41	**0.000**	21.15	37.82	**0.000**	19.88	41.05	**0.000**	0.69	2.08	0.904
NXH	-	-	**0.019**	-	-	**0.008**	-	-	0.870	-	-	0.346	-	-	0.819

For nitrogen (N): C_1 and C_2 represent relative changes against N_{125} (125% of recommended N) and N_{150} (150% of recommended N), respectively

For herbicide (H): C_1 and C_2 represent relative changes against H_{1000} (1000 ml ha^{-1}) and H_{2000} (2000 ml ha^{-1}), respectively

The bold values are significant at $P < 0.05$

Table 6 Relative changes (%) in available nitrogen, available phosphorus, available potassium, dehydrogenase activity (DHA), fluorescein diacetate hydrolyzing capacity (FDA), acid phosphatase activity (ACP), and alkaline phosphatase activity (AKP) in soil due to application of nitrogen and penoxsulam at different growth stages of rice

Treatments/stage	Available nitrogen			Available phosphorus			Available potassium			DHA			FDA			ACP			AKP		
	C_1	C_2	P	C_1	C_2	P	C_1	C_2	P	C_1	C_2	P	C_1	C_2	P	C_1	C_2	P	C_1	C_2	P
Tillering																					
N_{100}	5.76	11.74	**0.043**	14.67	34.46	**0.001**	11.49	19.43	**0.017**	14.76	19.36	**0.001**	28.31	58.79	**0.000**	4.17	14.62	**0.046**	8.63	14.70	**0.027**
H_0	1.62	2.47	0.826	5.80	10.53	0.287	0.62	3.29	0.820	−3.62	−5.19	0.375	0.37	1.41	0.963	−8.86	−7.29	0.187	−1.37	−1.90	0.909
NXH	-	-	0.961	-	-	0.995	-	-	0.987	-	-	0.366	-	-	0.318	-	-	0.652	-	-	0.603
Panicle initiation																					
N_{100}	5.30	16.79	**0.032**	21.05	45.17	**0.000**	9.39	19.57	**0.011**	3.62	12.66	**0.034**	16.15	20.74	**0.007**	5.29	28.93	**0.000**	6.49	48.00	**0.000**
H_0	9.49	15.57	0.053	5.15	13.85	0.091	7.20	14.83	0.050	−6.12	−22.41	**0.000**	−1.86	−35.68	**0.000**	−5.57	−21.16	**0.001**	−5.87	−24.22	**0.000**
NXH	-	-	0.849	-	-	0.903	-	-	0.186	-	-	0.903	-	-	0.956	-	-	0.840	-	-	0.712
Anthesis																					
N_{100}	6.57	12.79	**0.036**	27.68	58.11	**0.000**	8.55	12.81	**0.027**	11.67	15.52	**0.007**	18.62	38.37	**0.000**	3.97	19.91	**0.001**	11.07	32.07	**0.000**
H_0	1.87	4.39	0.599	8.49	11.53	0.166	6.96	8.72	0.127	−6.27	−9.97	0.051	−0.95	−2.61	0.670	−7.73	−8.22	0.089	−6.93	−6.98	0.241
NXH	-	-	0.999	-	-	0.581	-	-	0.349	-	-	0.523	-	-	0.924	-	-	0.179	-	-	0.374
Maturity																					
N_{100}	11.71	27.19	**0.000**	19.13	53.59	**0.000**	11.50	16.01	**0.031**	8.10	9.01	0.066	10.90	13.91	0.153	−0.20	13.76	0.051	9.91	17.35	0.055
H_0	3.82	4.61	0.570	7.24	12.78	0.116	2.97	7.98	0.341	0.93	3.24	0.678	-1.04	-3.26	0.877	−4.54	−11.62	0.126	−0.12	−0.82	0.989
NXH	-	-	0.329	-	-	0.335	-	-	0.590	-	-	0.509	-	-	0.933	-	-	0.060	-	-	0.470

For nitrogen (N): C_1 and C_2 represent relative changes against N_{125} (125% of recommended N) and N_{150} (150% of recommended N), respectively

For herbicide (H): C_1 and C_2 represent relative changes against H_{1000} (1000 ml ha^{-1}) and H_{2000} (2000 ml ha^{-1}), respectively

The bold values are significant at $P < 0.05$

is also supported by Peng et al. (2017) who reported that the application of additional nitrogen fertilizer boosted the concentrations of available forms of nitrogen, such as NH_4^+ and NO_3^- ions. These findings also suggest that there is a positive influence of applied nitrogen on the size of the soil microbial population which further causes mineralization of the organic nitrogen present in the soil. However, it was lower at maturity probably due to the uptake of nutrient by plants and several associated phenomena leading to losses of nitrogen. At higher doses of N, the availability of phosphorus increases as found in the study which is supported by the fact that ammonium ion effect favors $H_2PO_4^-$ availability. Ammonium ion markedly increases the solubility of dicalcium phosphate. Another possible explanation would be that the increase in P solubility is caused by the decrease in pH of submerged soils caused by flooding especially the solubility of hydroxyapatite increases as pH lowers (Stumm and Morgan 1970). There is an increment in the availability of K in response to higher doses of N, and this finding is supported by Stehouwer and Johnson (1991). As the K^+ and NH_4^+ ions have similar characteristics like charge, size, and hydration energy, they have strong interactions in soil. In the 2:1 clay minerals' interlayers and edges of interlayers, both ions are held by the same non-exchangeable sites. As a result, a straightforward competition should be predicted, with one ion displacing the other and increasing its percentage in the soil solution. However, the reality is more complicated, because applying NH_4^+ or K^+ to soil can result in both increases and decreases in the counter ion's non-exchangeable pool.

Penoxsulam application in soil at recommended dose insignificantly decreased all the four enzymatic activities such as DHA, FDA, ACP, and AKP as found in the study. But there is a significant decrease found in the corresponding values under double the recommended dose of the penoxsulam. This phenomenon may be attributed to the fact that the increased dose of herbicide may inhibit the microbial population present in the soil. Jabusch and Tjeerdema (2006) reported that DHA was not affected by the application of penoxsulam (40 g a.i. ha^{-1}) in rice-flooded soil. Raj et al. (2021) also reported that penoxsulam in combination with cyhalofop butyl applied in direct-seeded rice did not have any inhibitory effect on DHA in soil. Alkaline phosphatase activity was found higher in the studied soil as compared to the acid phosphatase which may be attributed to the inherent pH-dependent characteristic of the particular soil. In the case of nitrogen application, all the four activities were found to be increased with higher doses of the nutrient. Sharma et al. (2020) have reported similar findings where the combined application of nitrogenous fertilizer and rice straw incorporation significantly increased the microbial biomass carbon (MBC) and basal soil respiration (BSR) due to enhanced soil enzymatic activities viz. DHA, FDA, and AKP. All the four enzyme activities were found minimum at maturity of rice which may be due to lower active root mass present in the soil in the terminal stage of the crop.

Application of N fertilizer as well as penoxsulam did not significantly alter the total content of N, P, K, Fe, and Zn as found in the study. This particular phenomenon may be attributed to increased plant biomass which could be the result of high nitrogenous fertilizer use and controlled weed population in the herbicide-treated plots. The overall percentage of these nutrients in rice grain might not be altered significantly. Amylose content was found to be decreased with the increase of nitrogen which was evidenced in another study conducted by Tumanian et al. (2020). The possible explanation may be due to higher activity of α-amylase at increased nitrogen level which led to the degradation of amylose in grains (Liang et al. 2021). Penoxsulam application in rice had significantly increased amylose content in grains. It may be due to the fact that penoxsulam could substantially increase phenolic contents and, thereby, antioxidant activity in rice grains which in turn inhibits α-amylase activity (Aleixandre et al. 2022). It is being reported that grains having low amylose content shall produce a high Glycemic Index (GI) which is not preferable for the consumers (Dipnaik and Kokare 2017). On the contrary, the use of penoxsulam at recommended dose may be beneficial as it could enhance the antioxidant activity of grains and thus lowering the GI ratio.

5 Conclusion

As per the findings, the study hypothesis is found valid and acceptable because excessive use of nitrogen fertilization and penoxsulam had a significant impact on the biochemical characteristics of rice, its yield, and soil enzyme dynamics. Among the biochemical parameters measured in leaf, the interaction of nitrogen and penoxsulam affected chlorophyll content at the panicle initiation stage and proline in the mid-tillering stage of rice. It is noteworthy that penoxsulam application improved total phenolic content, amylose content, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity in rice grains signifying increased health benefits. Most importantly, the prerequisite for any suitable recommendation should assure production sustainability and improved soil health. According to this study, rice field could benefit from using 125% nitrogen coupled with the appropriate dosage of penoxsulam (H_{1000}), since this practice increased yield level over 100% nitrogen and decreased under 150% nitrogen level. With the application of 125% nitrogen, the available nutrients were maintained and soil enzymatic activities in soils were improved, demonstrating the ability to maintain a favorable environment for soil biological functions related to nutrient dynamics.

It could be conferred that a slightly higher dose of nitrogen fertilizer could lessen the possible plant harm caused by the toxic effects of penoxulam, resulting in a good yield. Consensus should be developed among the farmers to restrain themselves from indiscriminate use of inputs like nitrogen fertilizer and penoxsulam, as it could have an inverse relationship with financial and environmental benefits in the long run.

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Author Contribution Rehan Reza: He has done the entire research work

Pritam Ganguly: Conceptualization of the work thought; manuscript preparation

Swaraj Kumar Dutta: Field management

Anupam Das: Data analysis

Shweta Shambhavi: Plant and soil nutrient analysis

Rajiv Rakshit: Conceptualization of the work thought; soil enzyme analysis

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Data Availability The primary data is available with the corresponding authors and can be shared on reasonable request.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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