Original Research Article

Biochemical Defenses Induced by Natural Silicon in the Management of Rice Yellow Stem Borer, *Scirpophaga incertulas* (Walker)

ABSTRACT

Rice (*Oryza sativa* L.) one of the primary and staple food crops consumed throughout Asia belongs to Poaceae family. Yellow stem borer, *Scirpophaga incertulas* W. is a predominant insect pest infesting rice in India which damages the crop throughout the cropping season.

Aim: Present investigation was carried out to study the efficacy of foliar sprayednatural silicon in inducing biochemical defenses and reducing the damage caused by yellow stem borer in rice.

Study design: Field experiment was laid out by using Randomized Block Design consisting of three different treatments replicated seven times.

Place and Duration of study: The experiment was carried outat Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during the year 2022.

Methodology: Field experiment was carried out to study the effect of natural silicon on per cent damage caused by rice yellow stem borer. In addition, enzyme studies were carried out to find the biochemical changes occurred in the plants due to different treatments. Treatments comprised of foliar spray of 2% naturalsilicon (100% SiO_2)at 15, 30 and 45days after transplanting(DAT) (T_1), TNAU organic package of practices for rice (T_2) and untreated control (T_3).

Results: Foliar spray of 100% pure natural silicon along with stem borer feedingresulted in significant increase in the activities of defense associated enzymes namely, peroxidase (PO), polyphenol oxidase (PPO) and phenylalanineammonia lyase (PAL). In addition to defensive enzymes, total phenol content was found to be significantly higher in silicon treated plants when compared to other two treatments. Such increase in biochemical parameters *viz.,* the defensive enzymes andphenols resulted in significant reduction in the damage caused by stem borer with the lowest mean of 2.78 %, followed by 6.32 % in organic practice as against 9.34 % in untreated control.

Conclusion: Silicon obtained from natural sources can be employedas a safe and promising organic amendment in the management of yellow stem borerinfesting rice by inducing resistance in plants.

Keywords:Rice, yellow stem borer, defensiveenzymes, phenols, dead heart, white ear

1. INTRODUCTION

Rice (*Oryza sativa* L), as the single most important human energy source, provides food to about half of the world's population [1]. Rice production plays a vital role in the economic development of many countries, and any crises that decrease the production of this commodity can adversely affect these countries. In almost all rice -producing countries of the world, insect pests and crop diseases are considered the major factors that contribute to a decrease in rice production. Studies have proved that in almost all rice producing countries including India, insect pests and crop diseases are taken as major factors that contribute to a decrease in rice production[2].More than 100 different insect species are known to be insect pests of rice of which about twenty are considered to be the major pests [3]. Yellow stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera; Pyralidae) is one of the most serious insect pests causing crop losses in all rice growing regions of India [4]. The pest is accountable for yield losses ranging from 10 to 60 per cent [5, 6]. The boring and feeding of stem tissues by stem borer larva mainly resulted in production of dead hearts during vegetative stage and white ear heads during reproductive stage [7]. Stem tunneling by the larvae weakens the stem and reduces the flow of water, nutrients and metabolites breakage and lodging of plants as they mature. Because of its monophagous and confined feeding nature, yellow stem borer is considered as a challenging pest to manage even after repeated application of chemical pesticides. Therefore, there is a constant thrust for effective alternative technique for the management of this pest. One such choice is the use of abiotic elicitors like silicon (Si) to utilize the mechanism of induced resistance through phytohormone signaling pathways [8, 9]. Silicon can boost up the biochemical defenses that plants produce after being attacked by insects and resulting in enhanced production of defensive enzymes and secondary metabolites [10, 11]. As a result, silicon was found to lessen the vulnerability of the crop to pests and decrease the damage [12]. In India, only limited studies have been carried out to examine the impact of silicon obtained from the natural sources in the management of rice yellow stem borer and these studies have not been reported using 100% pure natural silicon in triggering biochemical defenses. Inducing plant's resistance is considered as potential alternative to chemical pesticides and as an effective, eco-friendly way to reduce insect pest populations. Keeping these in mind, it was decided to look into how the biochemical defense in plants can be enhanced and the incidence of *S. incertulas* would be affected by foliar application of natural sources of pure silicon.

2. MATERIALS AND METHODS

2.1. Field experiment

Studies on efficacy of natural silicon in the management of rice yellow stem borer, *S. incertulas* was carried out at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during the year 2022. The experiment was laid out using Randomized Block Design (RBD) with three treatments and replicated seven times with the rice variety CO 55. Seedlings were raised in the nursery for 25 days and were transplanted in the experimental plots, each measuring 5 m x 4.2 m at the rate of 2 seedlings per hill. The transplanted seedlings were maintained organically with a hill to hill spacing of 20 x 20 cm. Main and sub channels were formed to irrigate the plots individually and bunds were raised between each plot to compare the effects of different treatment.

T_1 - Foliar spray of pure natural silicon (Mex Mining, Hyderabad) @ 2% at 15, 30 and 45 days after transplanting.

T_2 - TNAU organic package of practices developed by Nammazhvar Organic Farming Research Centre, Tamil Nadu Agricultural University, Coimbatore with basal application of neem cake @ 250 kg/ha and neem seed kernel extract 5%.

T_3 - Untreated control

Observations on damage caused by yellow stem borer were recorded by counting the total number of tillers and tillers with dead heart or white ear symptoms at 15, 30, 45, 60, 75 and 90 days after transplanting. Based on these counts, per cent dead hearts and white ear heads were calculated as described by Jeer *et al.*[13] and mean damage was worked out.

$$\text{Percent dead hearts} = \frac{\text{Total number of dead hearts}}{\text{Total number of tillers}} \times 100$$

$$\text{Percent white ears} = \frac{\text{Total number of white ears}}{\text{Total number of productive tillers}} \times 100$$

2.2. Laboratory experiments

Laboratory experiments were carried out at Department of Biochemistry, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India to assess the activities of defensive enzymes *viz.,* peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL). In addition, the activity of secondary metabolite, total phenols was also estimated. All these biochemical parameters were analyzed by taking the fourth expanded leaves from ten randomly selected plants from each experimental plot at 50 days after transplanting.

2.2.1 Estimation of polyphenol oxidase (PPO)

Activity of the enzyme polyphenol oxidase was estimated using the methoddescribed by Augustin *et al.* [14] with slight modifications. Leaf samples weighing 0.5 gram collected from each treatment were homogenized using a pre-chilled pestle and mortar by adding 5 ml of 0.1 M sodium phosphate buffer (pH 6.5). The homogenate was centrifuged at 3,000 rpm for 15 minutes at 4 °C and the filtered supernatant was used as enzyme source for the assay of polyphenol oxidase activity. Standard reaction mixture consisted of 2 ml of 0.1 M sodium phosphate buffer (pH 6.5), 0.5 ml of the enzyme preparation and 0.3 ml of 0.1 M catechol. During the assay, buffer alone was taken as a blank. The reaction mixture was incubated at 28±1 °C and absorbance was set to zero at 495 nm in the spectrophotometer. The changes in the optical density were followed at an interval of 30 seconds for 3 minutes and the PPO activity was expressed as change in the optical density of the reaction mixture per minute per gram on fresh weight basis.

2.2.2 Estimation of peroxidase

The activity of peroxidase was estimated as per the procedure described by Hammerschmidt *et al.*[15] with slight modifications. The enzyme extract was prepared by using the same protocol adopted for PPO estimation. The assay mixture for estimation consisted of 2.5 ml of 0.1 M sodium phosphate buffer (pH 6.5), 0.1 ml of 0.05 M pyrogallol, 0.2 ml of enzyme extract and 0.2 ml of 0.2 M hydrogen peroxide. At the beginning of the enzyme reaction, the absorbance of the mixture was set to zero at 430 nm in the spectrophotometer and changes in the absorbance was recorded at 30 seconds interval for 3 minutes. All the procedures were carried out under 0-5°C. During the assay, water was run as a blank. Peroxidase activity was expressed as change in the absorbance of the reaction mixture per minute per gram on fresh weight basis.

2.2.3 Estimation of phenylalanine ammonia-lyase (PAL)

Phenyl alanine ammonia lyase content was estimated as per the method suggested by Rao and Towers [16] with slight modifications. From each rice plot, 0.5 gram of leaf samples were collected and homogenized in a pre-chilled pestle and mortar with 5 ml of 0.1 M sodium borate buffer (pH 8.8) containing a pinch of polyvinyl pyrrolidone. The homogenate was centrifuged at 12,000 rpm for 20 minutes. The supernatant was used for the assay of phenylalanine ammonia lyase activity. Phenylalanine ammonia lyase activity was determined at 290 nm using spectrophotometer by the conversion of L-phenyl alanine to trans-cinnamic acid at 30 °C. The reaction mixture consisted 0.5 ml of 0.2 M sodium borate buffer (pH 8.7), 0.5 ml of the enzyme extract and 1 ml of 0.1 M L-phenyl alanine (pH 8.7). The pH of L-phenyl alanine was adjusted by adding few drops of 0.1 N KOH. The blank was run by taking 2.5 ml of 0.1 M sodium borate buffer (pH 8.8) and 0.5 ml of the enzyme extract. The reaction mixture and the blank were incubated at 40 °C for 30 minutes and the reaction was stopped by adding 0.5 ml of 1 M Trichloro acetic acid (TCA). The absorbance read at 290 nm in a spectrophotometer. A standard graph was generated using various concentrations of trans-cinnamic acid. The PAL activity was expressed in moles of cinnamic acid produced per minute per gram on fresh weight basis.

2.2.4 Estimation of total phenols

The total phenols were estimated by using Folin-Ciocalteu reagent as per the method described by Malik and Singh [17]. Leaf samples weighing 250 mg collected from each of the experimental plots were homogenized with 10 ml of 80% ethanol and centrifuged at 10,000 rpm for 20 min and the supernatant was collected. The residue was re-extracted and the supernatants were pooled and the volume made up to 15 ml. Aliquots of 0.1 ml of each sample were pipetted out separately in different test tubes and the volume made up to 3.5 ml using distilled water. The tube with 3.5 ml of distilled water alone was taken as blank. After that, 0.5 ml of Folin-Ciocalteau reagent was added to each tube including blank and the tubes were allowed to stand for 5 minutes. Later, 2 ml of 20 per cent sodium carbonate was added and stirred. At 650 nm, the absorbance was measured against blank. A standard curve was generated using various concentrations of pyro-catechol. From the standard curve, the concentration of phenols in the leaf samples were determined and expressed as mg equivalent of pyrocatechol / 100 g of sample.

2.3 Statistical analysis

The data obtained from both laboratory and field experiments were subjected to analysis of variance (ANOVA) and analyzed using AGRES ver. (7.01), Pascal international solutions[18]. Least Significant Difference (LSD) was used to differentiate the means of significantly different treatments and the level of significance was fixed at P = 0.05.

3. RESULTS AND DISCUSSION

3.1. Effect of natural silicon on the damage caused by yellow stem borer

The results of field experiment conducted during 2022-2023 revealed that, at 15 days after transplanting (DAT), dead heart in the field ranged from 5.79 to 6.03%, with the lowest damage of 5.79% in TNAU organic package treated plots. At 30 DAT, the damage was recorded to be the minimum in the plots treated with foliar spray of 2% natural silicon (3.74 per cent), followed by TNAU organic package treated plots (6.83 per cent) as against the maximum of 7.18 per cent in untreated control. At 45 DAT also, similar trend was noticed with the minimum dead heart damage of 2.36 per cent in T_1 as against 9.03 per cent in untreated control. At 60 DAT, the plots treated with 2% natural silicon recorded the lowest damage of 1.97 per cent white ears, followed by TNAU organic package (6.54 per cent) as against 9.49 per cent in untreated control. Similar trend was noticed at 75 and 90 DAT also, with the minimum damage of 1.34 and 1.21 per cent white ears in T_1, as against 11.43 and 12.94 per cent white ears in untreated control, respectively. Mean damage caused by yellow stem borer in the form of either dead heart or white ears was found to be the lowest (2.78 per cent) in the plots treated with 2% natural silicon at 15, 30 and 45 DAT, followed by 6.32 per cent damage in the plots treated with TNAU organic package of practices. While, maximum mean damage of 9.34 per cent was recorded in untreated control (Table 1). The results of the present study were in accordance with the previous study. Foliar spray of orthosilicic acid at the rate of 4 ml/l was found to be highly effective in reducing damage incurred by yellow stem borer [19]. Additionally, soil application of one of the silicon sources, rice husk ash was found effective to reduce the damage caused by *S. incertulas* in rice under field conditions [13]. The yellow stem borer damage results in dead heart leading to death of the plant. In the current study the application of pure silicon resulted in better management of this pest in rice. Research revealed that the application of inorganic silicon source *viz.,*calcium silicate at 2 t/ha and organic silicon source, diatomaceous earth at 0.45 t/ha reduced significantly dead heartsand white ears caused by yellow stem borer and leaf damage caused by rice leaf folder [18].Besides, Hosseini et al. [20] found significant reduction in white ear percent in Parto cultivar (0.11 %) when 20 g of silicon was applied per kg of soil against striped stem borer in rice grown under greenhouse conditions. Yellow stem borer is difficult to manage even after the repeated insecticidal applications due to its cryptic behaviour and concealed feeding habut and moreover there is no resistance variety present for this pest. The application of organic forms of silicon *viz.* diatomaceous earth at 400 kg/ha and rice husk ash at 2 t/ha were found to be effective in inducing resistance in rice plants against yellow stem borer Panda et al. [21]. The results of the present study revealed that silicon treatment registered 70.24 per cent reduction in stem borer damage over untreated control as against 32.33 per cent reduction in TNAU organic package of practices treatment. The results are in-line with the findings of Chandramani et al. [22] who reported that split or basal application of combination of organic amendments such as neem cake, lignite fly ash and biofertilizers such as silicate solubilizing bacteria, azospirillum and phosphobacterium significantly reduced the incidence of stem borer, leaf folder and gall midge in rice.

3.2. Effect of natural silicon on the activity of defensive chemicals in rice

Activities of plant defensive enzymes *viz.*, PAL, PPO and PO were found to be significantly increased in silicon treated samples (0.91, 0.22 and 0.59 change in absorbance/min/g, respectively), followed by TNAU organic package of practices treated plants (0.18, 0.10 and 0.41 change in absorbance/min/g, respectively). In contrast, the activity of all the three defensive enzymes was significantly reduced in untreated control (0.08, 0.06 and 0.36 change in absorbance/min/g) as shown in Table 2. From Figure 2, it is observed that foliar application of 2% natural silicon registered maximum activity of the enzyme phenyl ammonia lyase, followed by peroxidase and polyphenol oxidase. Leaf samples treated with TNAU organic packages had better enzymatic activities when compared to untreated control. The results were in accordance with findings of Karban and Myers [23] who reportedelevated activity of defense responses in plants, notably peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase, which attributed to the activation of resistance. Han et al. [24] had proven that application of silica at the rate of 0.32 g/kg soil along with leaf folder infestation notably increased the levels of PO, PPO and PAL. They also demonstrated that silica amendment induced the resistance mechanism in rice plants against leaf folder. Gomes et al. [25] reported that application of calcium silicate at the rate of 1.855 g/kg of soil combined with green bug infestation in wheat resulted in induction of resistance by significantly increasing the activities of PO, PPO and PAL and decreasing the population of aphids.

Peroxidases play a major role in the suberization and lignification processes [26, 27]. As an outcome of the oxidation of phenolic substances to quinones by the polyphenol oxidase, the nutritional value and protein digestibility of food get diminished [28, 29]. An increase in the levels of phenylalanine ammonia-lyase in injured plant tissues implies an increased rate of the production of phenolic compounds which are widely recognized as the plant’s defense molecules against infections and insects [30].In this study total phenol content was also found to be significantly increased in silicon treated plants, followed by TNAU organic practices and untreated control (0.36, 0.23 and 0.17 mg of pyrocatechol / 100 g, respectively) (Figure 3). Similar results were reported by Padhi who stated that enhanced production of silica and phenolic compounds increased the resistance in rice plants and reduced the damage caused by borers [31].

In addition to exogenous application of silicon, damage caused by yellow stem borer was also an important factor in enhancing the biochemical based defensive activities. It was corroborated by the findings of Amsagowri et al. [32] who reported that rice plants infested by stem borer significantly elevated the levels of ortho-dihydroxy phenols and total phenols under glasshouse conditions. They also reported that such increase in biochemical activities attributed towards the resistance against yellow stem borer. The authors also stated that infestation of rice plants by different insect pests *viz.,* yellow stem borer, leaf folder and brown plant hopper showed a significant increase in the quantity of phenols. Such enhanced production of defensive compounds resulted due to the stress signaling mechanism in plants regulated by phytohormone pathways [33].

4. CONCLUSION

The study gave a vital importance of pure natural silicon in the management of rice yellow stem damage. The pure natural silicon treatment provided better results in reducing dead heart and white ears as compared to the untreated control. Such application enhanced the production of biochemical defensive compounds namely PAL, PPO, PO and total phenols in rice against yellow stem borer. Hence, natural silicon can be recommended in the organic rice production system as a potential defensive source in the management of rice yellow stem borer.

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Table 1. Effect of natural silicon on the damage caused by yellow stem borer

Treatments	Yellow stem borer damage (%) (Dead heartor white ears)*							Per cent reduction over control
	15 DAT	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	Mean	
T_1 - Foliar application of 2%natural silicon	6.03[a]	3.74[a]	2.36[a]	1.97[a]	1.34[a]	1.21[a]	2.78	70.24
T_2 - TNAU organic package of practices	5.79[b]	6.83[b]	7.12[b]	6.54[b]	6.33[b]	5.28[b]	6.32	32.33
T_3 - Untreated control	5.96[a]	7.18[c]	9.03[c]	9.49[c]	11.43[c]	12.94[c]	9.34	-
S. Ed	**0.07**	**0.08**	**0.11**	**0.13**	**0.20**	**0.10**	**-**	-
CD (P=0.05)	**0.15**	**0.17**	**0.24**	**0.28**	**0.44**	**0.21**	**-**	-

**DAT - Days after transplanting; NS-Non significant; *Mean of 7 replications*
In a column, means sharing similar letter(s) is/are not significantly different by LSD at P=0.05%

Table 2. Effect of natural silicon on the activity of defensive chemicals in rice

Treatments	Total Phenols(mg/g)	Activity of plant defensive enzymes (changes in OD value/min/g of fresh leaf)		
		Phenyl Alanine Ammonia Lyase	Polyphenol oxidase	Peroxidase
T_1 - Foliar application of naturalsilicon @ 2%	0.36[a]	0.91[a]	0.22[a]	0.59[a]
T_2 - TNAU organic package of practices	0.23[b]	0.18[b]	0.10[b]	0.41[b]
T_3 - Untreated control	0.17[c]	0.08[c]	0.06[c]	0.36[c]
S. Ed	**0.02**	**0.03**	**0.01**	**0.02**
CD (P=0.05)	**0.04**	**0.07**	**0.03**	**0.05**

**Each value is the mean of seven replications*
In a column, means sharing similar letter(s) is/are not significantly different by LSD at P=0.05%

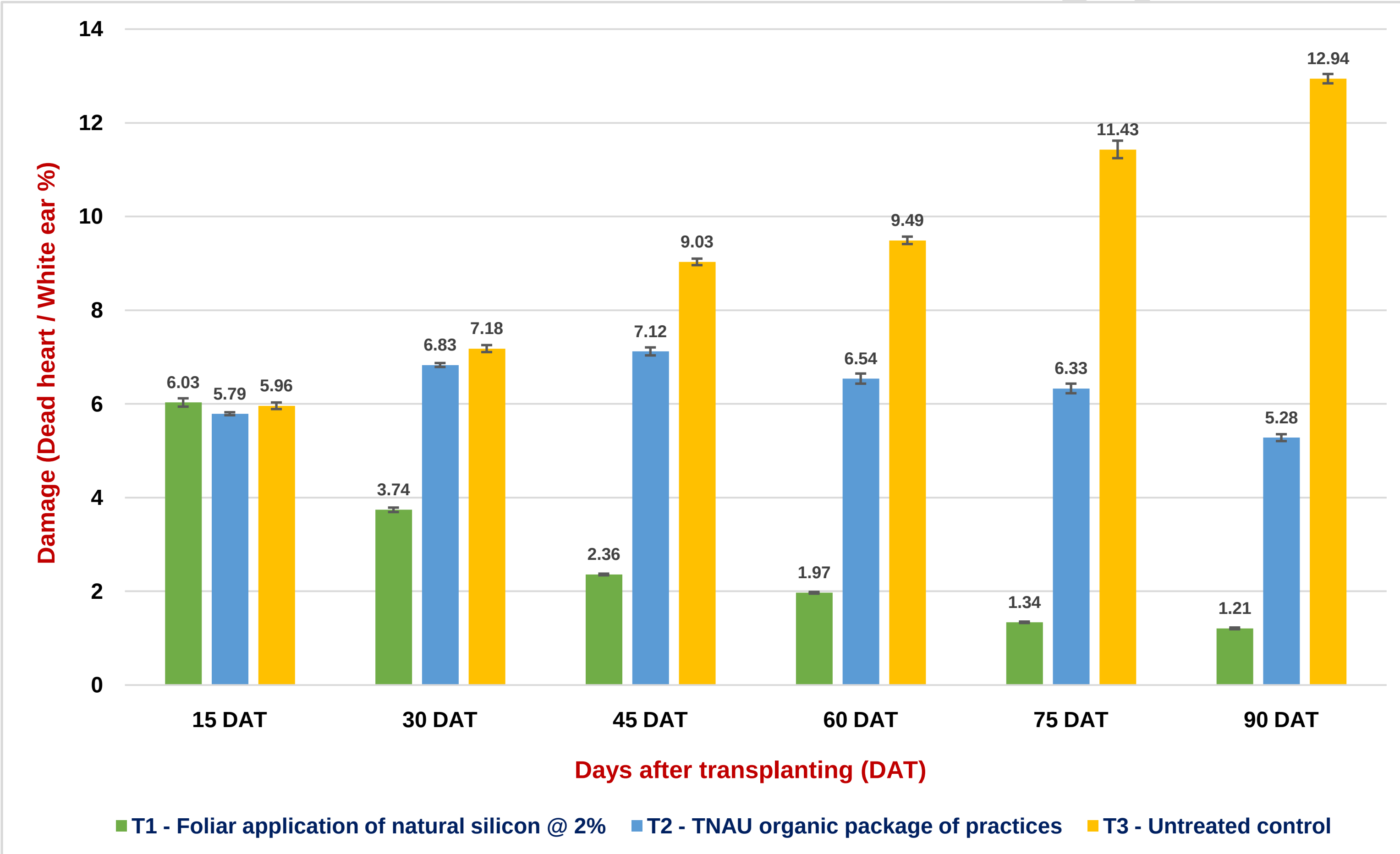

Fig. 1. Effect of different treatments on the damage caused by yellow stem borer

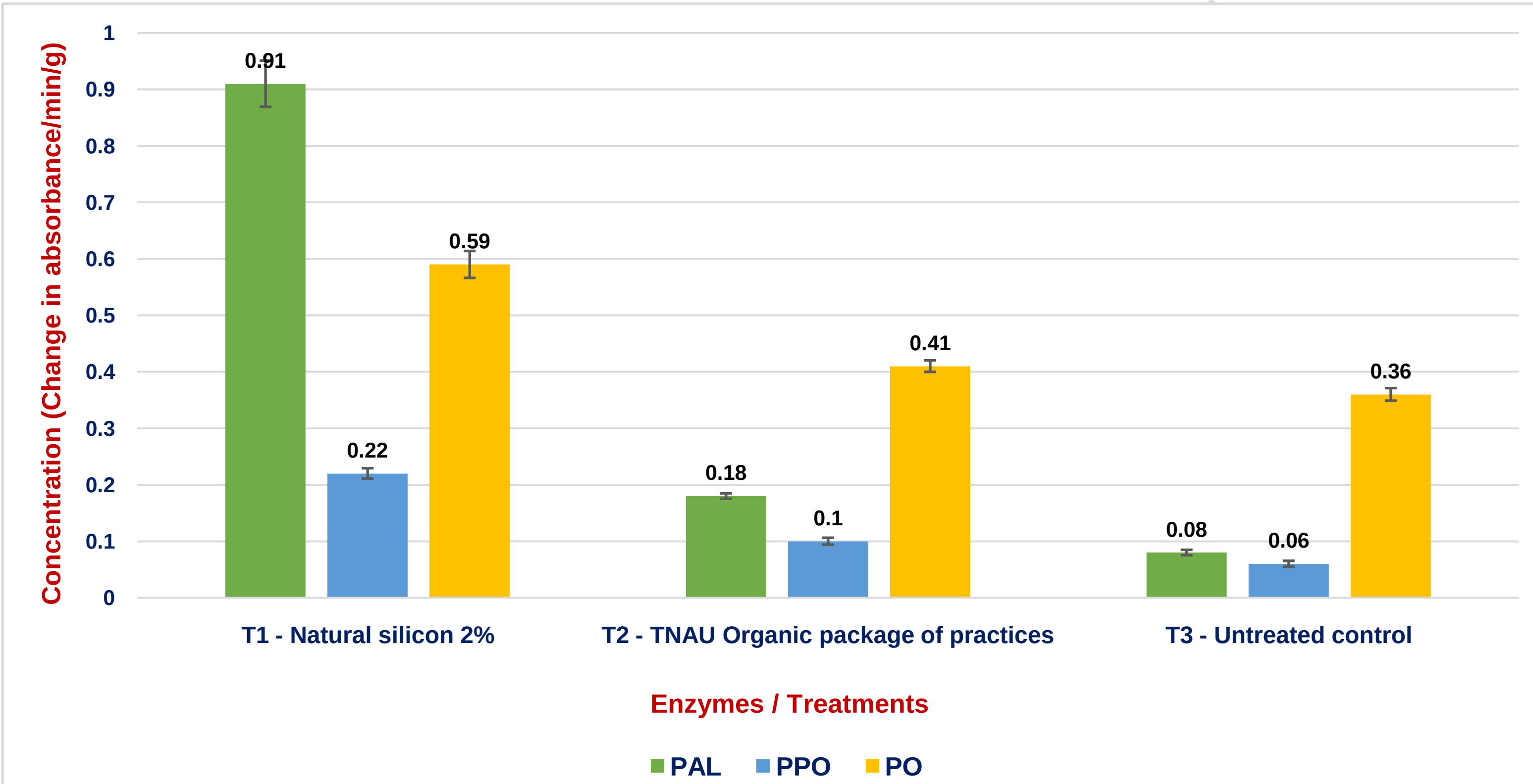

Fig. 2. Effect of different treatments on the activity of plant defensive enzymes in rice

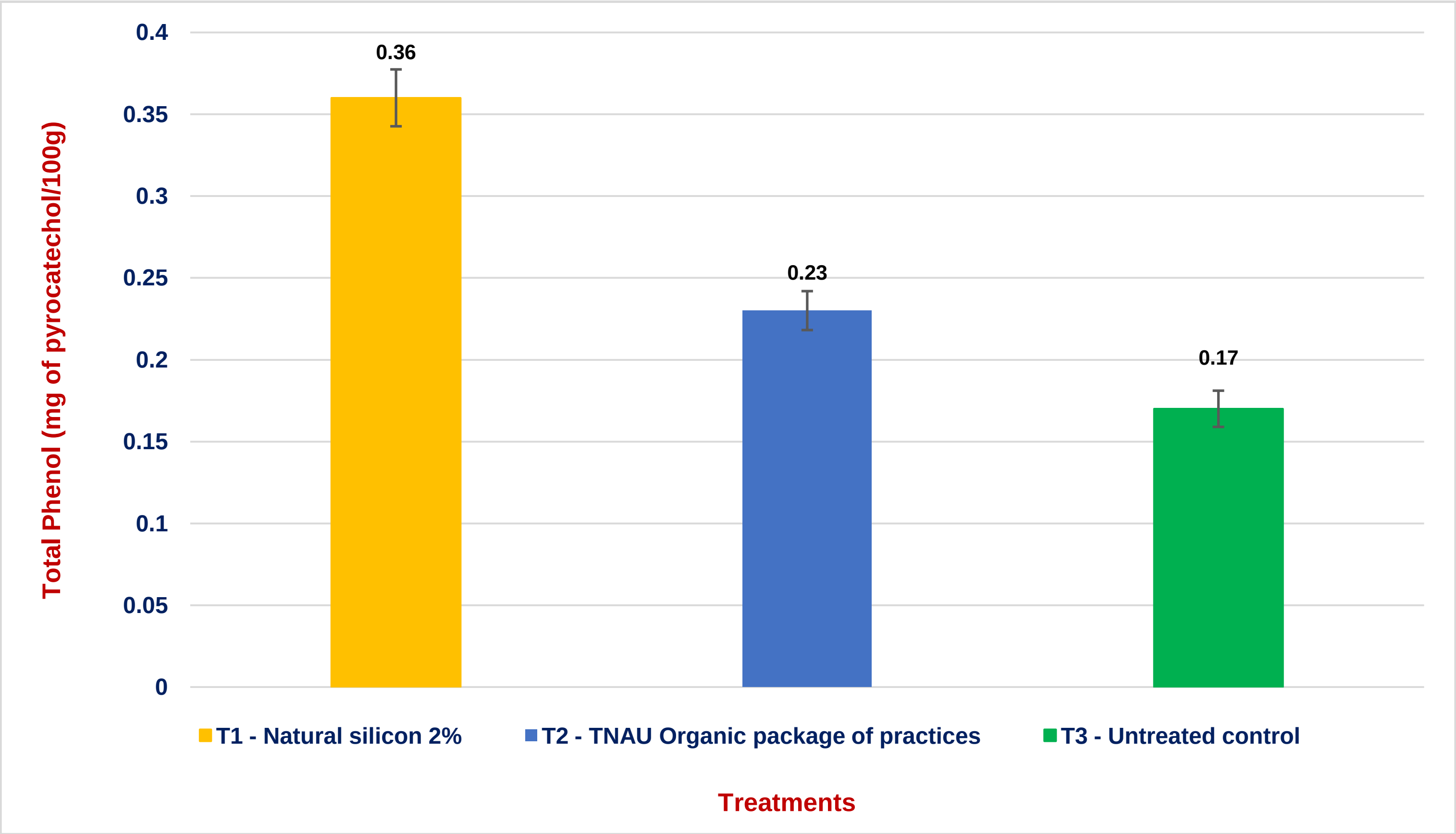

Fig. 3. Effect of different treatments on total phenol content in rice