The Pharma Innovation Journal 2023; 12(5): 1940-1945

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(5): 1940-1945

www.thepharmajournal.com
Received: 22-02-2023
Accepted: 25-03-2023

S Panda
Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

JJ Pastagia
College of Agriculture, Navsari Agricultural University, Waghai, Gujarat, India

DH Patel
Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

HG Patel
Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

SK Mahapatra
School of Agriculture, Gandhi Institute of Engineering and Technology University, Gunupur, Odisha, India

Corresponding Author:
S Panda
Department of Entomology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India

Silicon mediated alterations in plant defense against yellow stem borer, *Scirpophaga incertulas* (Walker) and secondary macro-nutrient uptake in rice

S Panda, JJ Pastagia, DH Patel, HG Patel and SK Mahapatra

Abstract
A study on impact of diatomaceous earth (DAE) and rice husk ash (RHA) on altering the plant defense against yellow stem borer in rice conducted at Navsari Agricultural University confirmed the resistance inducing ability of silicon in rice cultivar TN1 against *Scirpophaga incertulas.* Basal application of silicate fertilizers augmented the uptake of calcium (Ca), magnesium (Mg) and sulphur (S) by rice plants grown under biotic stress and establishing a negative correlation (r= -0.687) between the former two. Based on overall findings, application of DAE at 400 and RHA at 2000 kg/ha proved most efficacious in enhancing the plant resistance against *S. furcifera* and hence may be may be recommended for field use.

Keywords: Rice, *Scirpophaga incertulas,* silicon, secondary macro-nutrient

Introduction
Rice (*Oryza sativa* L.), the staple food of India, is widely consumed by over half of the world's population, especially in Asian and African countries. Most of the rice produce comes from Asia with China and India being the lead contributors. India, however, has almost half productivity as that of China and this yield gap can mainly be attributed to various biotic and abiotic stress during crop growth. Rice ecosystem harbors a diverse group of insect pests, amongst which the yellow stem borer (YSB) is the most important one and dominates in East and South-East Asia (Khan *et al*., 1991) [1], infesting at all stages of the crop (Bandong and Litsinger, 2005) [2]. The larvae contribute to yield loss by boring into the stem, resulting in dead heart at vegetative stage and white ear at heading stage. Depending on the crop growth stage, the yield loss could range from 10 to 90% (Muralidharan and Pasalu, 2006) [3]. Chemical control through foliar spray is relatively ineffective because of a narrow window of their exposure to insecticides prior to stem boring. Moreover, the indiscriminate use of insecticides has adversely affected the environment necessitating a sustainable and eco-holistic approach to contain the pest and minimize the yield loss. Recently, induction of resistance in rice plants with certain biotic and abiotic elicitors to tackle stress is being widely experimented. Silicon (Si) is one such elicitor, which has been reported to enhance level of resistance in rice crops against various stress (Alhousari and Greger, 2018) [4].
Calcium (Ca), Magnesium (Mg) and Sulphur (S) are essential secondary macro-nutrients chiefly responsible for cell structural integrity (Morgan *et al.*, 2014) [5], chlorophyll synthesis (Führs and Gerendás, 2013) [6] and amino acid synthesis (Rahman *et al*., 2007) [7], respectively. Evidences on Si fertilization affecting the uptake of these nutrients exist (Greger *et al*., 2018 [8]; Shen *et al.*, 2009 [9], Korndorfer *et al*., 2001) [10], which in turn influence the plant defence system indirectly. This study aims at understanding the role of Si and biotic stress in altering the plant defence mechanism through change in uptake of secondary macronutrients.

Materials and Methods
Pot culture experiment was undertaken in *kharif* 2021 at Navsari Agricultural University, Navsari in Complete Randomized Design with 11 treatment combinations comprising two organic sources of Si *viz*., Diatomaceous Earth (DAE) at 100, 200, 300, 400, 500 kg/ha and Rice Husk Ash (RHA) at 1000, 1500, 2000, 2500, 3000 kg/ha along with an untreated check. Two sets of treatments were maintained with and without YSB infestation to ascertain the impact of biotic stress along with Si on uptake of macro nutrients and their role in defending YSB damage in rice. Pots of 12" diameter were filled with 10 kg of soil and treatments were imposed through basal soil application before planting.

Ten-day-old petri-plate germinated seedlings of susceptible rice variety TN1 were transplanted at the centre of each pot (one seedling per pot) and immediately covered by Mylar cage to protect the seedlings from outside pest attack. For imposing biotic stress in a separately maintained set, five freshly hatched YSB larvae, obtained through laboratory rearing were released at 20 days after transplanting (DAT). Stem borer damage was assessed by counting the total tillers and dead heart (DH) at 7 and 14 days after infestation (DAI) from infested plants. After crop maturity plants were harvested, oven dried at 70 ºC for 72 hours, grinded to fine powder and stored for laboratory use.

One gram of sample was subjected to tri-acid digestion by mixing 5ml nitric acid in a conical flask, keeping it for overnight and then putting on hot plates for heating at 100 ºC till acid gets completely evaporated. After cooling, 10ml of nitric acid: perchloric acid (3:2) was added and then heated till the sample gets charred. The content was then cooled, dissolved with 5ml of hydrochloric acid and then filtered into a 100ml volumetric flask using Whatman No. 42 filter paper. The volume of the filtrate was then made up with double distilled water. This sample extract was diluted 10 times and used in ICP-OES (Inductively Coupled Plasma- Optical Emission Spectroscopy) for estimation of Si, Ca, Mg and S content. Data on per cent borer damage were square root transformed prior to subjecting to statistical analysis for test of significance. Nutrient uptake data were presented graphically with standard error bar and compared with that of Si content.

Results and Discussion

Plant defense mechanism against stress is a complex mechanism. Silicon confers resistance to host plants against biotic stress by improving the physical rigidity of tissues, exhibiting antibiosis defense, priming biochemical defense, triggering defense related genes, affecting nutritional uptake by plants and acceleration of the release of plant organic volatiles to attract natural enemies (Alhousari and Greger, 2018 [4]; Islam *et al*., 2020) [11]. The three secondary macronutrients (Ca, Mg and S) along with silicon, have been reported to have a critical role in plant defense against stress. A number of studies showed that Si plays a vital role in regulating the nutritional uptake and maintaining a balance in plants during the stress and non-stress periods (Marschner 1995 [12]; Waraich *et al*., 2011) [13].

Silicon and *S. incertulas* damage

Data presented in Table 1 revealed the silicon mediated response of rice plants to YSB damage at the vegetative stage of the crop. Application of both the organic silicate fertilizers were effective in containing the borer damage. Seven days after infestation (DAI) stem borer damage was minimal in plants receiving DAE at 400 kg/ha and RHA at 1500 and 2000 kg/ha with a record of 13.33-18.88% DH as against 41.11% DH in control. Typically, at the highest doses, both the sources failed to restrict the pest damage with 27-30% DH. After two weeks DAE performed better at 300-500 kg/ha doses registering 20.4- 25.5% DH and remained on par with that of RHA 2000 kg/ha (24.6%) as against 44% in control. Overall performance indicates greater efficacy of DAE at 400 kg/ha and RHA at 2000 kg/ha in arresting the borer damage. Low borer damage in various treatments corresponds to greater Si accumulation with a record of 8.40-9.58% as against 4.92% in control, which suffered the most from borer attack (Fig 1). This greater accumulation of Si probably creates a physical barrier causing decreased borer penetration resulting in lower stem damage. Such resistance to borers leads to lengthening the larval stage thus, exposing themselves to more predation (Hou and Han, 2010) [14]. Si amendments leading to low success of penetration by first instar larvae by Asiatic rice borer has been reported by Hou and Han (2010) [14]. Effective reduction in stem borer infestation in rice attributing to various mechanisms has also been reported earlier by Chandramani *et al*. (2009) [15]; Hou and Han (2010) [14]; Pati *et al*. (2016) [16], which corroborates the present findings. Low efficacy of DAE and RHA at their higher doses has also been reported earlier by Mishra *et al.* (2018) [17]; Panda *et al*. (2022) [18] and the reason for which needs a thorough investigation.

Table 1: Stem borer damage in susceptible and resistant rice varieties as influenced by sources and doses of silicon under pot culture

Treatment details		Stem borer damage at vegetative stage (%DH)				Si content (%)
Tr. No.	**Source & Dose**	**7 DAI**	**14 DAI**	**Mean**	**% Decrease over control**	
T1	DAE @ 100 kg/ha	33.03 (5.78)	37.05 (6.12)	35.04	17.67	5.450
T2	DAE @ 200 kg/ha	30.03 (5.51)	33.26 (5.80)	31.65	25.64	5.425
T3	DAE @ 300 kg/ha	22.53 (4.78)	23.81 (4.91)	23.17	45.59	9.575
T4	DAE @ 400 kg/ha	15.63 (3.99)	20.37 (4.56)	18.00	57.75	8.400
T5	DAE @ 500 kg/ha	27.27 (5.25)	25.46 (5.08)	26.37	38.06	6.850
T6	RHA @ 1000 kg/ha	24.00 (4.94)	36.24 (6.06)	30.12	29.24	5.238
T7	RHA @ 1500 kg/ha	18.18 (4.31)	29.64 (5.48)	23.91	43.85	5.788
T8	RHA @ 2000 kg/ha	13.33 (3.71)	24.64 (5.01)	18.99	55.42	6.475
T9	DAE @ 2500 kg/ha	16.67 (4.11)	25.03 (5.04)	33.37	21.59	6.688
T10	DAE @ 3000 kg/ha	30.00 (5.11)	31.43 (5.64)	30.72	27.83	6.570
T11	Control	41.11 (6.44)	43.99 (6.67)	42.55	0.00	4.925
SE(m)±		0.274	0.228			
$CD_{0.05}$		0.80	0.67			
CV%		8.63	6.99			

Figures in parenthesis are √(x+0.5) transformed values

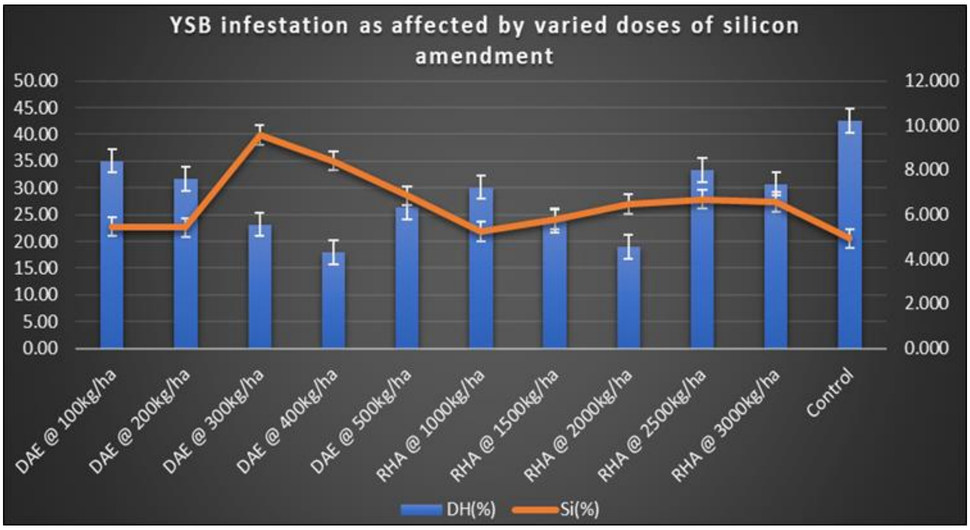

Fig 1: Yellow stem borer damage in rice as affected by varied sources and doses of silicon

Silicon and Nutrient uptake

Calcium (Ca)

The study revealed a differential impact of sources of silicon on Ca uptake by rice plant tilting in favour of RHA with a visible increase over control more specifically under biotic stress. On the other hand, DAE resulted in declined Ca uptake either with or without biotic stress. RHA at higher doses receiving YSB feeding stimuli resulted in enhanced uptake of Ca with a record of 0.175-0.22% compared to 0.127-0.176% concentration in plants without biotic stress, as against a corresponding figure of 0.176 and 0.179% in control (Fig. 2). Several literatures support the positive correlation between Si and Ca uptake in various crops grown under any kind of stress (Kaya *et al.*, 2006) [19] or in optimal conditions (Mali and Aery, 2008 [20]; Gottardi *et al.*, 2012 [21]; Greger *et al.*, 2018 [8]). However, a decrease in calcium content in rice shoots with the addition of silicon was earlier reported by Ma and Takahashi (1993) [22] which was observed in DAE treated plants. Overall finding of this study suggests a positive response of Si in increased uptake of Ca only under biotic stress. This finding is supposed to be advantageous in tackling the YSB damage in a better way through use of Si.

Calcium plays an essential role in forming structural and functional integrity of membranes, cell stability, ion homeostasis (Arshi *et al.*, 2010 [23]; Morgan *et al.*, 2014 [5]). The

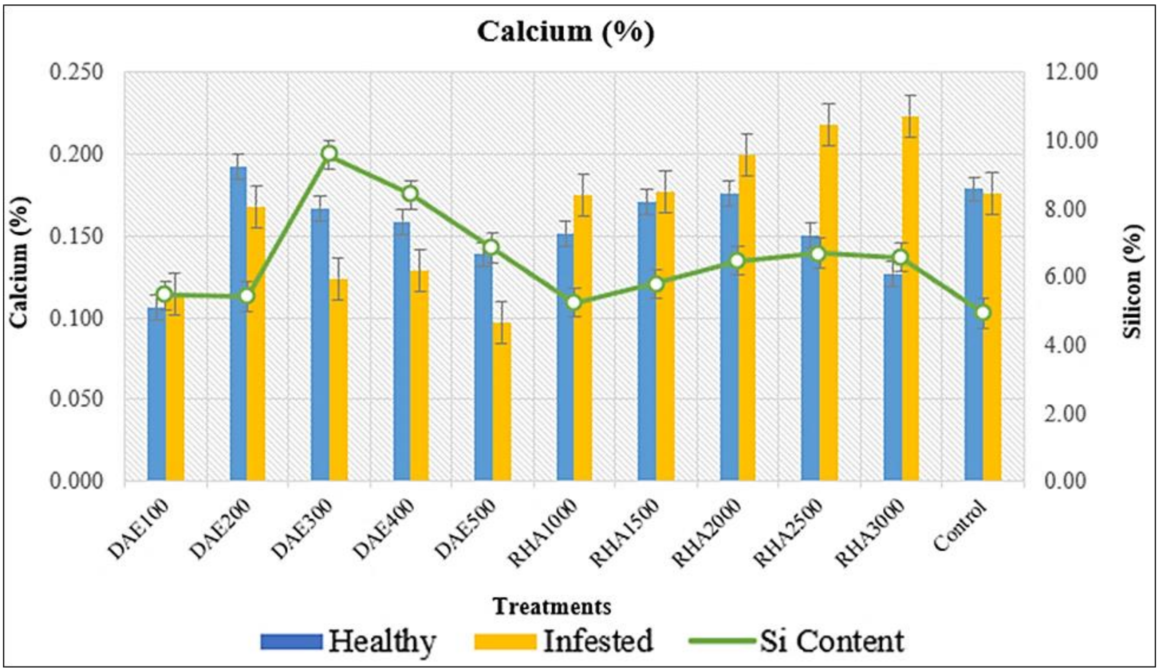

Fig 2: Calcium content in rice plants treated with DAE and RHA in presence or absence of biotic stress

formation of calcium silicate double layer in epidermis acts as a physical barrier to the YSB larvae as has been reported earlier (Nikpay *et al.*, 2015) [24]. According to Rahman *et al.* (2016) [25] intensification of antioxidant enzymatic system and reactive oxygen species detoxification system is being facilitated by calcium in plants, which adds to its role in enhancing plant defence mechanism to biotic stress. Furthermore, Calcium (Ca^{2+}) is believed to be acting as an essential plant element and acts as signalling molecules associated with adaptive responses against environmental stresses (Mahmood-ur-Rahman *et al.*, 2019) [26], that probably strengthened the plant defence mechanism against YSB.

Magnesium (Mg)

Irrespective of biotic stress, the DAE treated plants registered a higher magnesium content compared to that of RHA amended plants (Fig. 3). An increased net accumulation of Mg with the addition of Si in various plant species was reported earlier by Greger *et al*. (2018) [8]. Plants infested with YSB invariably showed a higher magnesium content in silicon amended plants, except those treated with RHA @ 1000 kg/ha the highest being in plants receiving DAE @ 500kg/ha (0.171%). However, under chromium stress, a decrease in Mg content has earlier been reported by Tripathy *et al*. (2012) [27], which was of course increased when plants were amended with Si. This study revealed that unlike abiotic stress, the YSB feeding increased the Mg content in plants devoid of externally applied silicon. As evident from the figures (Fig. 2 and 3), both Mg and Ca were negatively related to each other establishing a negative correlation (r = -0.687*) with each other. Magnesium is involved in many physiological and biochemical process of a plant ensuring its proper growth and

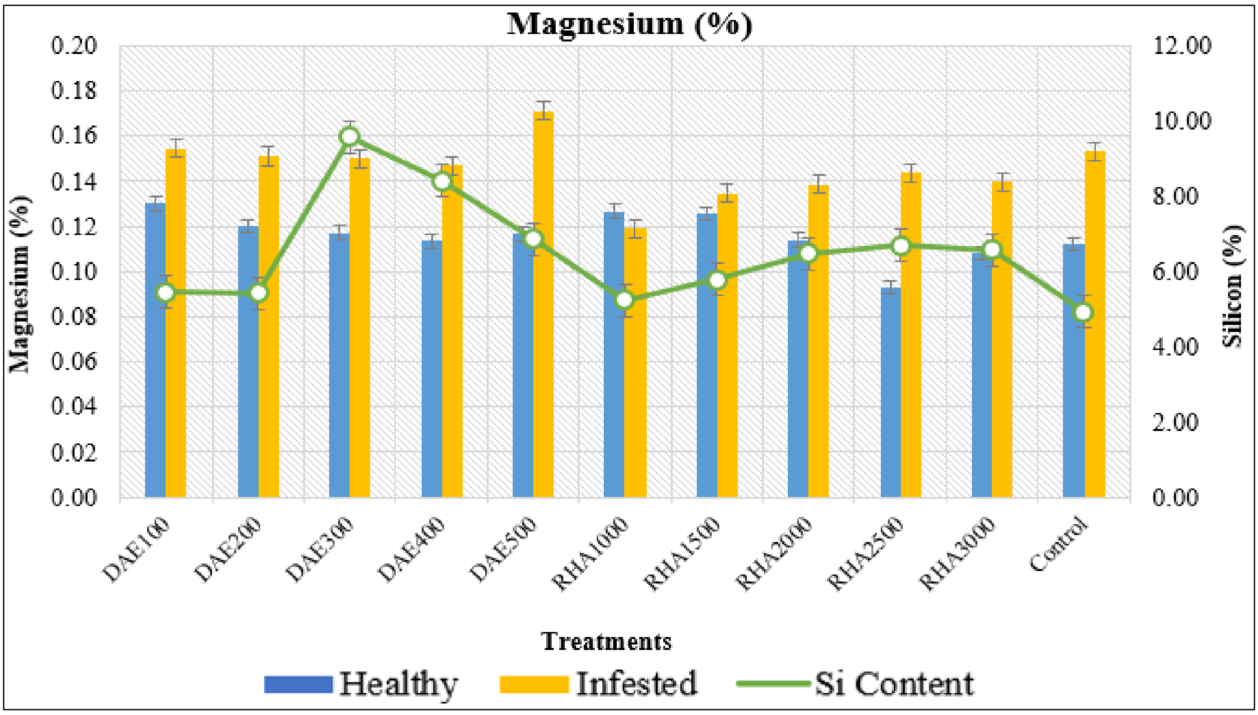

Fig 3: Magnesium content in rice plants treated with DAE and RHA in presence or absence of biotic stress

development. Mg has been proved to have a role in plant defense mechanisms against stress situations (Cakmak and Kirkby, 2008 [28]; Cakmak and Yazici 2010 [29]; Cakmak 2013 [30]; Führs and Gransee, 2013 [6]; Huber and Jones 2013 [31]; Mengutay *et al*. 2013 [32]). Further, alleviation of Mg deficient induced stress in silicon treated plants by restoring chlorophyll levels and improving shoot fresh weight was observed by Hosseini *et al*. (2019) [33] signifying the role of Si in Mg deficient soil contributing indirectly to plant defence against the herbivore pests.

Sulphur

The current studies revealed an increased accumulation of sulphur due to Si addition, which was prominent in plants receiving DAE and with biotic stress (Fig. 4). However, healthy plants responded positively to higher doses (2000-3000 kg/ha) of RHA and showed a sharp rise in S uptake, thus revealing impact of interaction effects of biotic stress and Si source. Under control situation (without Si amendment) however, YSB infestation resulted in sharp increase in S content, which is probably due to the response to the feeding stimuli from insect. Increase in S uptake in rice facilitated by silicon fertilisation was reported earlier by Korndorfer *et al*. (2001) [10] and Singh *et al*. (2006) [34]. Increased sulphur content in silicon unamended (control) infested plants (0.608%) as against un-infested plants (0.080%) highlighted the role of stress in triggering the process of accumulation of sulphur in rice plants. This increase in S content due to Si application is supported by the earlier findings of Patel *et al*. (2019) [35] who reported a positive correlation between both the elements. The role of S in improving rice productivity, synthesis of chlorophyll, amino acids like methionine, cystine, cysteine and some plant hormones such as thiamine and biotin has been well documented by Rahman *et al*. (2007) [7], which indicates its probable role in indirect strengthening of the plant defense. system.

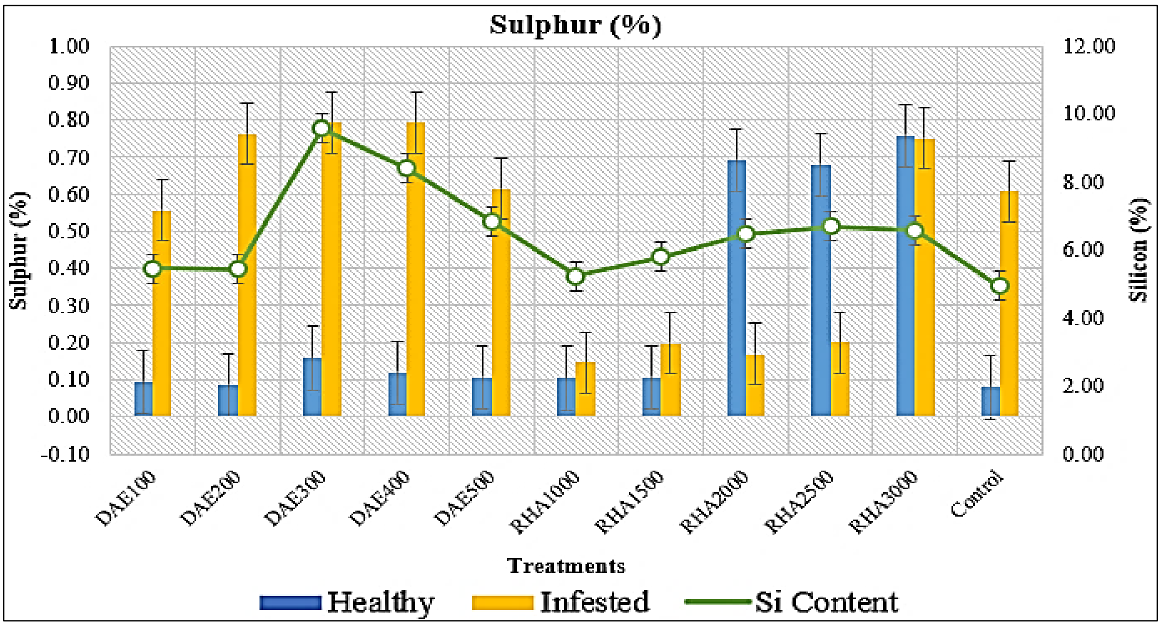

Fig 4: Sulphur content in rice plants treated with DAE and RHA in presence or absence of biotic stress

Conclusion

The study confirmed the promising effect of Si amendment through organic sources like diatomaceous earth and rice husk ash with maximum performance at 400 and 2000 kg/ha in inducing host plant resistance against rice yellow stem borer. Silicon supplements resulted in higher uptake of Ca, Mg and S specifically under biotic stress and are supposed to play a vital role in enhancing plant defense against rice yellow stem borer. Based on overall findings, and considering the cost, ease of availability and environmental impact, application of RHA, an organic souse of Si may be recommended at 2.0 t/ha in rice as a pest management strategy.

Acknowledgements

The authors would like to thank Department of Science and Technology (Government of India) for funding this research through INSPIRE Fellowship (2018) and Odisha University of Agriculture and Technology, Bhubaneswar) for access to ICP-OES to conduct nutritional analysis.

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