

Seed treatment as a preventive strategy for *Nephotettix virescens* (Distant) management in rice cultivation

Tapon Kumar ROY^{1*}, Anamika SANNAL², Mst. Hasna HENA¹, Sanjida AKTER¹, Jannatul Yeasmin JOATY¹, Mir Md Moniruzzaman KABIR¹, Md Najmul BARI¹, Abu NAYEEM¹, ABM Anwar UDDIN¹, Sania TAMANNA¹, Md Mosaddek HOSSAIN¹

¹ Entomology Division, Bangladesh Rice Research Institute, BRRI, Gazipur 1701, Bangladesh; tapon.entom@brrri.gov.bd; hasna.brrri43@gmail.com; sanjida.bgr@gmail.com; jannatuly.bsmrau94@gmail.com; mkabir1985@gmail.com; nbarientom@gmail.com; abun4386@gmail.com; masumbrrri@gmail.com; saniatamanna635@gmail.com; agmosaddeque@gmail.com

² Department of Plant Pathology Division, Gazipur Agricultural University (GAU), Gazipur 1706, Bangladesh; sannalanamika9@gmail.com

* Corresponding author: tapon.entom@brrri.gov.bd; taponroy.brrri@gmail.com (Bangladesh)

Abstract: Early-stage infestation of green leafhopper (GLH) in rice often requires foliar insecticides application, which can adversely affect natural enemies and the environment. This study investigated the efficacy of seven insecticides applied as seed treatment using a completely randomized design. Seed treatments significantly influenced seed germination, with the maximum germination observed in the untreated control (92.00%) and the minimum in curtap (63.33%). Among the tested insecticides, imidacloprid & thiamethoxam (neonicotinoids) performed excellent germination rates (>86.33%) and enhanced seedling vigor; imidacloprid displayed the longest root (5.15 cm) & shoot (4.15 cm) length. The maximum damage of seedling was exhibited in the untreated control under both choice (50.67%) and no-choice (94.67%) tests, whereas imidacloprid (choice:6.00%, no-choice:0.33%) and thiamethoxam (choice:7.00%, no-choice:3.67%) provided strong protection against GLH infestation. Neonicotinoid treatments consistently suppressed GLH populations and reduced F₁ generation development, while untreated control demonstrated the highest densities. The maximum prevalence of GLH, rice whorl maggot, brown planthopper, zigzag leafhopper was observed in untreated control, curtap and isoprocarb plots but minimum at neonicotinoid plots. The abundance of natural enemies harboring maximum in untreated plots, while spider population was maximum under untreated and imidacloprid plots, suggesting lower non-target impacts. Overall, thiamethoxam and imidacloprid demonstrated the most effective for suppressing GLH population and boosting seedling vigor, while being compatible with some natural enemies; highlighting their potential for reducing reliance on foliar sprays.

Keywords: rice; seed treatment; imidacloprid; neonicotinoids; thiamethoxam; rational insecticide use

Received for publication on November 20, 2025

1. Introduction

Rice (*Oryza sativa* L.; Family: Poaceae) is staple cereal crop that is extensively cultivated in many countries and about one-third of the world's population mostly depends on it. Rice is one of the major crops in Bangladesh, which is grown extensively throughout the country all year round. It plays the role of staple food in Bangladesh and is necessary to ensure food security. There are three rice growing seasons in Bangladesh, namely- Aman (monsoon rice), Boro (irrigated rice) and Aus (pre-monsoon rice) which covers the yearly rice productions. But, the rice productions is hampered by various factors, including selection of variety, management practices, biotic and abiotic stressors and environmental conditions. Infestation of insects in rice fields is common problem during rice cultivations. The abundance of rice insect pests are frequently presence from seedling to harvesting stage of rice. There are 232 species of harmful insects and 375 species of natural enemies are closely associated with rice eco systems of Bangladesh (Islam & Catling, 2012; Roy et al., 2025a; Roy et al., 2024). According to (Islam & Catling, 2012; Roy et al., 2025a; Roy et al., 2024), the green leafhopper (*Nephotettix virescens* Distant), the brown planthopper (*Nilaparvata lugens* Stal^o), the yellow stem borer (*Scirpophaga incertulas* Walker), the dark headed borer (*Chilo suppressalis* Walker), the rice leafhoppers (*Cnaphalocrocis medinalis* Guenée), the white-backed planthopper (*Sogatella furcifera* Horváth), the rice hispa (*Diuraphis armigera* Oliver) and the gall midge (*Orseolia oryzae*, Wood-mason) are the major pest species in Bangladesh.

Among these, the infestation of the green leafhopper (GLH) is very common in all rice ecosystem of Bangladesh (Islam & Catling, 2012; Roy et al., 2024). The adults of GLH are highly mobile and when disturbed they fly away. The female of GLH lays eggs (8-16) in batches which are arranged in parallel in a small slit on the leaf sheath inner surface (Islam & Catling, 2012). They normally prefer young plant for egg laying. Nymphs of GLH prefer to feed on the dorsal surface of the leaf blades and from the mid portions of leaves (Islam & Catling, 2012). GLH often feed and appear in the upper plant canopy, and causes direct damage by sap sucking behavior and plugging the vascular bundles from leaf (Islam & Catling, 2012). When GLH largely feeds on vascular bundles and sucks the sap, then the rice plants seem to die, it is known as "hopperburn". But hopperburn does not occur all time, rather it transmits viral diseases "tungro" (Islam & Catling, 2012; Roy et al., 2024). Both adults and nymphs carry virus vector responsible for tungro disease. Tungro infected plants have deformed leaves, stunted growth, gall formation or yellowing and increased tillering which causes total yield losses (Islam & Catling, 2012). Although the abundance of GLH is found year round, but the distinct population peaks in the reproductive phase of rice (Islam & Catling, 2012; Roy et al., 2024). Moreover, one of the main reasons of insect pest migration and infestation from one rice season to another rice season is the overlapping of rice seasons in Bangladesh (Bari et al., 2025; Islam & Catling, 2012; Roy et al., 2024). The Aus season expands from mid-March to August, whereas the Aman season is from July to November and from mid-November to mid-May is the expansion period of the Boro season in Bangladesh. Additionally, April-June and October- November are the peak prevalence periods of GLH (Bari et al., 2025; Islam & Catling, 2012; Morshed et al., 2023; Roy et al., 2024). Therefore, in some parts of Bangladesh, severe infestations of GLH are found at early growth stage of rice.

Traditionally, manual foliar applications of chemical insecticides like organophosphates, pyrethroids, neonicotinoids, nereistoxin and diamides have been used to manage GLH in rice fields (Islam & Catling, 2012; Roy et al., 2025b; Shultana et al., 2025; Simon-Delso et al., 2015). However, synthetic pesticides have an impact on insect-pests like GLH and natural enemy populations

throughout the seedling and early stages of rice (Roy et al., 2025b; Tejaswi et al., 2024). It can be difficult to transfer pesticides from mother tiller to new tiller or from older leaves to younger leaves via foliar spray (Buchholz & Nauen, 2002; Roy et al., 2025b; Tejaswi et al., 2024). So, frequent spraying of insecticides is necessary in order to protect new tillers and young leaves, however, this increases the expense of crop production and is harmful to natural enemies and environment. Compared to foliar sprays, seed treatment with insecticides can provide a suitable alternatives to this problem, since insecticides on seeds can be continuously absorb and transmits to newer leaves due to upward conduction of insecticides (Alford & Krupke, 2017; Roy et al., 2025b). Therefore, a single seed treatment with little quantity of insecticide can prevent insect infestations during the seedling stage of rice. Seed treatments have been demonstrated to be a particularly successful and efficient technique that uses less pesticide overall due to the potency of neonicotinoid (Kundoo et al., 2018; Roy et al., 2025b). Therefore, in the field of crop protection, seed treatment emerged as new strategies. Seed dressing, seed coating and soil treatments are also considered to be much safer and can be able to replace foliar spraying with provides effective early stage protection against insect pests due to their systemic qualities which transfers all over the plant (Jeschke et al., 2011). Nowadays, treatments of seed and soil around 60% of all neonicotinoid applications are made to fight pests that infests crops including - rice, maize, wheat, potatoes, vegetables, sugar beet, cotton, and others (Jeschke et al., 2011). So, multi-layer coating of seed, pelleting, seed dressing and seed film coating are all shows excellent and secure ways to protect young plants from insects attack. However, there are very few literature available about seed treatment against green leafhoppers in rice. In Bangladesh, there were no study about seed treatment against GLH. Therefore, a comprehensive study is still require to determine how registered insecticides differs from other neonicotinoid insecticides which uses as seed treatments in terms of their efficacy in controlling *N. virescens* at an earlier stage of rice and their safety for natural enemies. So, the goal of our present study is to investigate the efficacy of various chemical insecticides at the early stage of rice growth parameters, to manage GLH and their effects on seed germination, development of root-shoot and natural enemies at the early stage of rice for the first time in Bangladesh.

2. Materials and Methods

2.1. Experimental site, materials and design

The experiment was carried out from August 2024 to April 2025 at green house facilities of entomology division, in Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh and research field of BRRI. The experiment used BRRI dhan89, a modern irrigated (Boro) rice variety that was gathered from the storage facilities of entomology division, BRRI. The seeds were thoroughly cleaned and sun-dried after being collected. In the greenhouse, a completely randomized design with three replications was employed. For the greenhouse condition, an aluminium tray of 185 cm in length, 100 cm in width, and 15 cm in height was utilized.

2.2. Rearing of Green Leafhopper

Green leafhopper (GLH) adults were collected from the research fields of BRRI. Following collection, the adults were placed in the metal framed rearing cage (100cm height × 160 cm length × 80 cm width) which metal net (mesh number 40) covering every side except the bottom side. The front side of rearing cage had an opening and shutting facilities for convenient operation. The BR3

(susceptible to insects) rice variety (tillering stage) was utilized as the GLH feeding plant. The feeding plant was supplied for GLH feeding and regularly renewed as needed. The feeding plants was cultivated independently in greenhouse with an insect free environment and without application of insecticides. The rearing process of GLH has continued until requirement. The greenhouse was maintained at a temperature of $27\pm 2^{\circ}\text{C}$ and a relative humidity of $70\pm 5\%$.

2.3. Experimental treatment and procedure

For this experiment, seven synthetic insecticides and one untreated control were used (Table 1). Eight earthen pots were taken and each earthen pot was filled with 1 L of fresh water. Synthetic insecticides were put according to table 1 in the earthen pot separately and then mixed carefully on 17th August 2024 for the 1st trial and 2nd February 2025 for the 2nd trial. After that, 500 g of BRRI dhan89 previously sundried and cleaned seeds then soaked with water within each earthen pot sequentially, and they were kept for 24 hours. For untreated control, only fresh water was used. Water solution from the pots were removed after 24 hours from the pot and the seed were transferred into germination chamber and kept for additional 72 hours to ensure proper germination. For the germination test and to measure the length of the roots and shoots, one hundred seed from each treatment were put in separate Petri dishes with moist filter paper at the bottom.

Table 1: Name and doses of different treatment with insecticide generic and group name for seeds treatment against GLH

Treatment	Insecticide generic name	Dose g/mL L ⁻¹	Name of the group
T1	Imidacloprid 70 WG	1.50 g	Neonicotinoid
T2	Curtap 50 SP	1.50 g	Nereistoxin analogues
T3	Isoprocarb 50 WP	1.50 g	Carbamate
T4 (Control)	Only fresh water	-	Untreated Control
T5	Tetrataniprole 20SC	1.50 mL	Diamides
T6	Cyantraniliprole 40 SC	1.50 mL	Diamides
T7	Thiamethoxam 25 WG	1.50 g	Neonicotinoid
T8	Buprofezin 40 EC	1.50 mL	Buprofezin

2.4. Preparations of soil and sowing of germinated seeds in greenhouse

Collections of soil were done from research field of BRRI, Gazipur to fill the aluminium tray at greenhouse conditions. Following collections, the soil was fully sundried, then mixed thoroughly with organic fertilizers and compost (soil and organic matter, 3:1 ratio) and after that, the mixed soil was made loosened and placed into the metal aluminium tray (length 185 cm × width 100 cm × height 15 cm). One aluminium tray was manually divided into four parts by soil bunds. Therefore, two aluminium trays were required for one sets of replication. After that, according to the experimental design the germinated seeds were shown on the tray. Two aluminium trays were placed side by side for one replication and the replication then covered by net (mesh number 40) on a metal structure. This procedure was known as the choice method. In a choice method, the green leaf hoppers were given simultaneous access to the insecticide-treated host plant material (e.g., all treated plants) and untreated (control) host plant material. It primarily assessed the GLH preference or rejection of the treated plant. If the insecticide is repellent or acts as an anti-feedant, the GLH will choose the other less toxic or repellent or untreated plant.

We also used no-choice methods technique. In a no-choice method, the GLH were confined to the insecticide-treated host plant material only (separate from one treatment to others), with no

alternative food source provided. It provided a rigorous measure of the insecticide's absolute toxicity, repellent and antifeedant activity or its physiological host range effect. Since the GLH is forced to be in contact with or feed only on the treated plant material, it determines if the insecticide will kill the pest even if the pest finds the treated plant undesirable. Furthermore, in the no choice method, germinated seeds were shown in small metal tray (length 40 cm × width 30 cm × height 15 cm) for single treatment. So, one complete replication required a total of eight trays. Each replicated aluminium tray had its own metal frame with a full net cover. The trays were set up in greenhouse conditions with a temperature of $27 \pm 2^\circ\text{C}$ temperature and a relative humidity of $70 \pm 5\%$.

2.5. Infestation of GLH under greenhouse conditions

Two to three instars of green leafhopper (GLH) nymphs were introduced @ 1000-1100 nymphs tray⁻¹ in choice methods (7 days after seeds sowing on tray and the newly emerged seedling had reached two leaves). But in no choice methods, GLH nymphs was introduced @300-350 nymphs tray⁻¹.

2.6. Seedbed preparation under research field and sowing of seeds in seedbed

The seedbed was prepared by ploughing following laddering in wet field of BRRI, Gazipur. Then, previously germinated seeds were sown into the moist seedbed (length 5 m × wide 1 m for one treatment) on 21 August 2024 for the 1st trial and 06 February 2025 for the 2nd trial. All treatments then replicated for three times.

2.7. Data collection

From the previous 100 separated seeds in Petri dishes, the germination percentage of seeds were determined at 7 days after soaking. From the same Petri dishes, the length of roots and shoots were also measured from 10 seedling. Similar process was applied for all replicated treatments. In both choice and no-choice method, the damage (%) of seedlings was computed by using a weekly basis random sampling of 100 seedlings and compared with (IRRI, 2013) and which were continued up-to 28 days of insect infestation and 35 days after sowing. Twenty seedlings were selected as random basis for counting the number of GLH in both choice and no choice methods. Five seedlings were selected randomly, put in a moist test tube which covered with a cotton ball, for counting the progeny of GLH emergence at 21 days after GLH infestations for one treatment. The appearance of GLH nymphs was then routinely monitored. All treatments and replications underwent this procedure. Moreover, in the field, sweeping was used once a week to collect insects from the seedbeds. The sweeping process began seven days after the seeds were sown and continued for up to eight weeks. Rectangular sweep nets (Kabir et al., 2023) were used to collect the seedbeds insects, which began on one side of a single seedbed and then returned in a similar manner from the opposite side. Following collections of insect-pests, they were kept in a deep freezer for ensuring death and then the numbers of insects and natural enemies were identified, counted and recorded. The entire process was carried out twice.

$$\text{Seed germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of tested seeds}} \times 100$$

$$\text{Seedling damage percentage} = \frac{\text{Number of damage seedlings}}{\text{Total number of counted seedlings}} \times 100$$

2.8. Statistical analysis

All data analysis and graphical representations were performed using RStudio software (version 2025.09.0+387). For illustrating graphs we used the "AgroR" package and for analysis of variance (ANOVA) we used the "doibioresearch" package for conducted to test for significance differences among treatment means. The normality of mean comparison (least significance difference) test was executed by Shapiro-Wilk Test, at 5% significance levels in RStudio software (version 2025.09.0+387).

3. Results

3.1. Effects of insecticides on germination percentage of seed

Insecticides impacted significantly on germination percentage of seed (Table 2). Statistically the highest seed germination (%) was exhibited at untreated control condition (92.00%), which was identical with tetrataniprole (89.00%) insecticide treatment. The lowest seed germination (%) was found at curtap (63.33%) treated seed and which was statistically different from other treatments.

3.2. Influences of insecticides on roots and shoots length of seedling

Insecticides significantly affected on root and shoot length of seedling (Table 2). The longest root length was demonstrated in the imidacloprid (5.15 cm) treatment, which was statistically identical to curtap (5.06 cm), followed by isoprocarb (4.43 cm) and untreated control (4.29 cm). Furthermore, the shortest route length among treatments was found at cyantraniliprole (3.24 cm) and which was statistically similar with tetrataniprole (3.42 cm), thiamethoxam (3.71 cm) and buprofezin (3.71 cm). Other-hand, the longest shoot length were observed at imidacloprid (4.15 cm), isoprocarb (3.62 cm) and untreated control (3.56 cm) treatments. The shortest shoot length was found at buprofezin (2.87 cm) treated insecticide treatment.

Table 2: Effects of insecticides on seeds germination (%), vigor of seedling, seedling damage (%) and number of F₁ population emergence of GLH

Treatment with insecticide generic name	Germination (%) of seed	Root length (cm) 7 Days after soaking	Shoot length (cm) 7 Days after soaking	Damage (%) of rice seedlings		Number of emergence 5-plants ⁻¹		F ₁
				Choice	No choice	Choice	No choice	
T1=Imidacloprid 70 WG	87.00 b	5.15 a	4.15 a	6.00 d	0.33 e	4.00 e	4.33 e	
T2=Curtap 50 SP	63.33 c	5.06 a	3.44 bcd	28.00 b	70.00 b	31.67 b	36.00 c	
T3=Isoprocarb 50 WP	88.00 b	4.43 b	3.62 ab	31.00 b	70.67 b	32.33 b	66.67 b	
T4=Untreated Control	92.00 a	4.29 b	3.56 abc	50.67 a	94.67 a	59.00 a	75.67 a	
T5=Tetrataniprole 20Sc	89.00 ab	3.42 c	3.13 bcd	26.67 b	51.33 c	26.33 bc	31.33 cd	
T6=Cyantraniliprole 40 SC	88.00 b	3.24 c	2.97 cd	17.33 c	27.00 d	18.67 d	25.00 d	
T7= Thiamethoxam 25 WG	86.33 b	3.71 c	3.43 bcd	7.00 d	3.67 e	8.33 e	5.67 e	
T8=Buprofezin 40 EC	86.66 b	3.71 c	2.87 d	31.67 b	50.00 c	25.00 d	35.33 c	
Levels of Significance	***	***	*	***	***	***	***	
Least Significance Difference	3.18	0.51	0.64	5.98	7.99	6.10	7.16	
Coefficient of Variation (%)	2.16	7.13	10.89	13.92	10.04	13.75	11.82	

3.3. Effects of insecticides on seedling damage (%) and number of F₁ population of GLH

3.3.1. Choice method

Insecticides affected significantly on seedling growth in both choice and no choice method. Statistically the maximum damage (%) of seedlings by GLH was observed at untreated control (50.67%) and the minimum damage (%) of seedling were found at imidacloprid (6.00%) and thiamethoxam (7.00) insecticide treatments (Table 2, Figure 1) in choice method. Statistically the

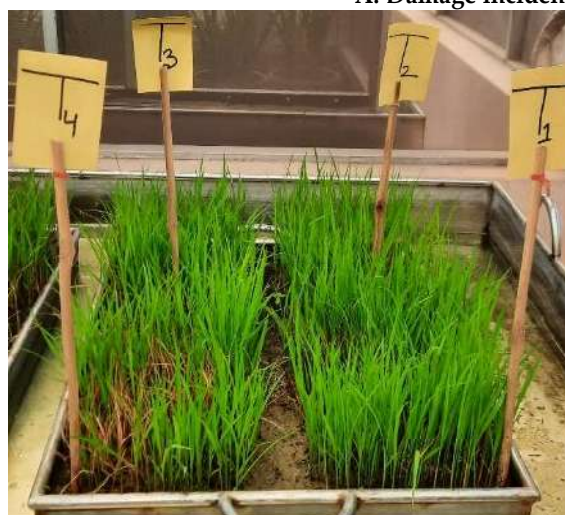
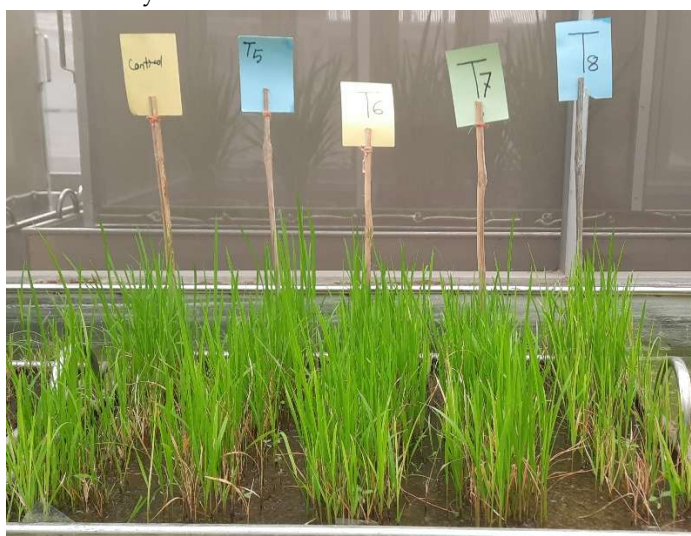
maximum number of F₁ GLH population was emerged at untreated control (59.00), which was followed by curtap (31.67), isoprocarb (32.33) and tetrataniprole (36.33) and they were statistically identical (Table 2). The insecticide treatment imidacloprid (4.00) and thiamethoxam (8.33) were exhibited the minimum number of F₁ GLH population and which were statistically similar.

3.3.2. No-Choice method

In no choice method, statistically the maximum damage (%) by GLH found at untreated control (94.67%), which was followed by curtap (70.00%) and isoprocarb (70.67%). Furthermore, the minimum damage by GLH was exhibited at imidacloprid (0.33%) which was statically identical with thiamethoxam (3.67%) treated insecticide (Table 2, Figure 1). Statistically the maximum number of F₁ GLH population was emerged at untreated control (75.67), which was followed by isoprocarb (66.67). The insecticide treatment imidacloprid (4.33) and thiamethoxam (5.67) were exhibited the minimum number of F₁ GLH population and which were statistically similar.

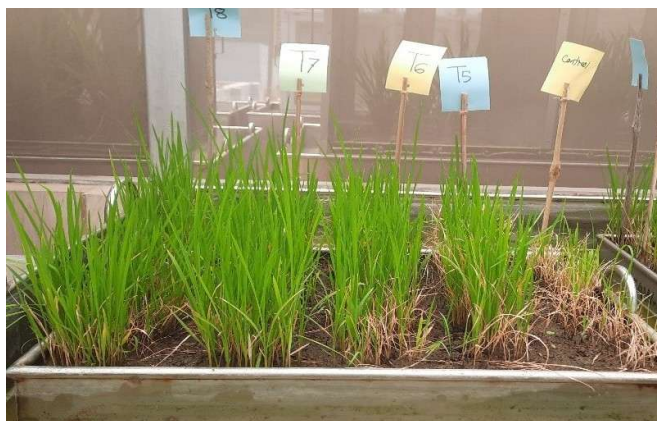


A. Damage incidence at 14 days after GLH infestation



B. Damage incidence at 21 days after GLH infestation





C. Damage incidence at 28 days after GLH infestation

Figure 1: Damage Incidence of green leaf hopper (GLH) at different treatments

Note: T₁= Imidacloprid 70 WG; T₂=Curtap 50 SP; T₃=Isoprocarb 50 WP; T₄=Untreated Control; T₅=Tetrataniprole 20Sc; T₆=Cyantraniliprole 40 SC; T₇= Thiamethoxam 25 WG; T₈=Buprofezin 40 EC.

3.4. Number of green leaf hopper 20-plant⁻¹

The number of GLH population recorded on seedlings treated with different insecticides showed significant difference at 7, 14, 21 and 28 days after infestation (DAI) (Figure 2 and Figure 3). At 7 DAI, the maximum number of GLH population were found at buprofezin (9.33) and which was significantly similar with tetrataniprole (8.00) and untreated control (9.00) condition (Figure 2A). While the minimum number of GLH population per 20 plant was demonstrated at imidacloprid (0.33) which was similar with thiamethoxam (1.33), cyantraniliprole (2.33) and curtap (2.33). Almost similar pattern followed for 14 DAI, 21 DAI and 28 DAI. At 14 DAI, the the maximum number of GLH population were found at untreated control (11.00) which was significantly similar with tetrataniprole (8.67) and followed by buprofezin (7.00). While at 14 DAI, the minimum number of GLH population per 20 plant was observed at imidacloprid (1.67) which was similar with thiamethoxam (1.67), cyantraniliprole (3.33), isoprocarb (4.00) and curtap (2.33) insecticide treatment (Figure 2B).

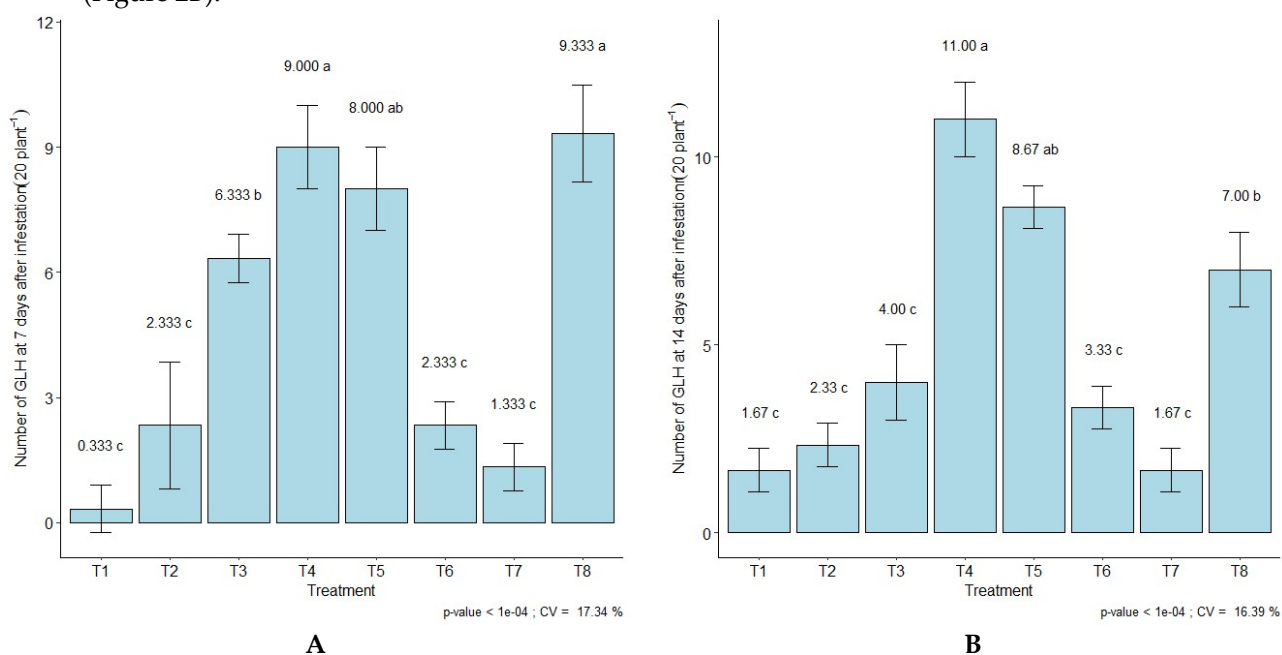


Figure 2: The number of GLH found 20seedlings⁻¹ at different days after infestation

At 21 DAI, statistically the maximum number of GLH population were exhibited at untreated control (9.67) which was significantly similar (Figure 3A) with tetrataniprole (8.00) and followed by buprofezin (7.067). In addition, the minimum number of GLH population per 20 plant was observed at imidacloprid (0.67) which was similar with thiamethoxam (1.67), isoprocarb (1.67) and curtap (1.33) insecticide treatment (Figure 3A). At 28 DAI, untreated control (12.00) showed highest number of GLH population per 20 plant (Figure 3B) where as imidacloprid (0.67) showed minimum population of GLH which was similar with thiamethoxam (0.67), isoprocarb (2.00), curtap (1.67) and cyantraniliprole (2.67). So, the average maximum number of GLH population per 20 plant was observed at untreated control (10.42). Imidacloprid (0.83) which was similar with thiamethoxam (1.33) were demonstrated the minimum number of GLH per 20 plant (Figure 4).

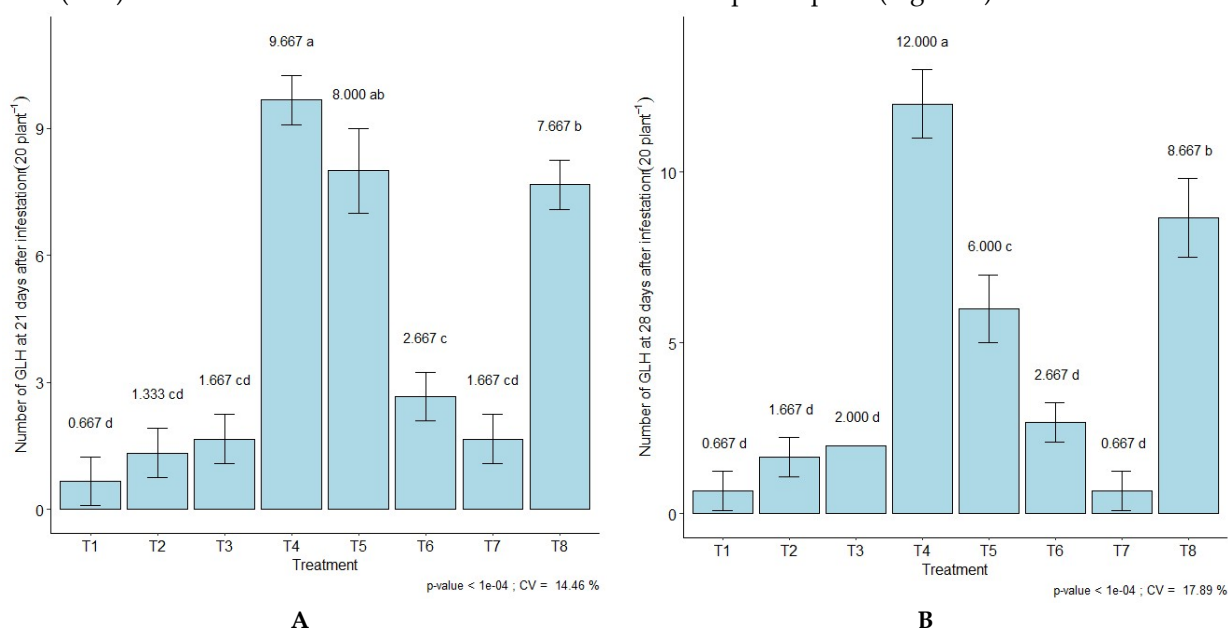


Figure 3: The number of GLH found 20seedlings⁻¹ at different days after infestation

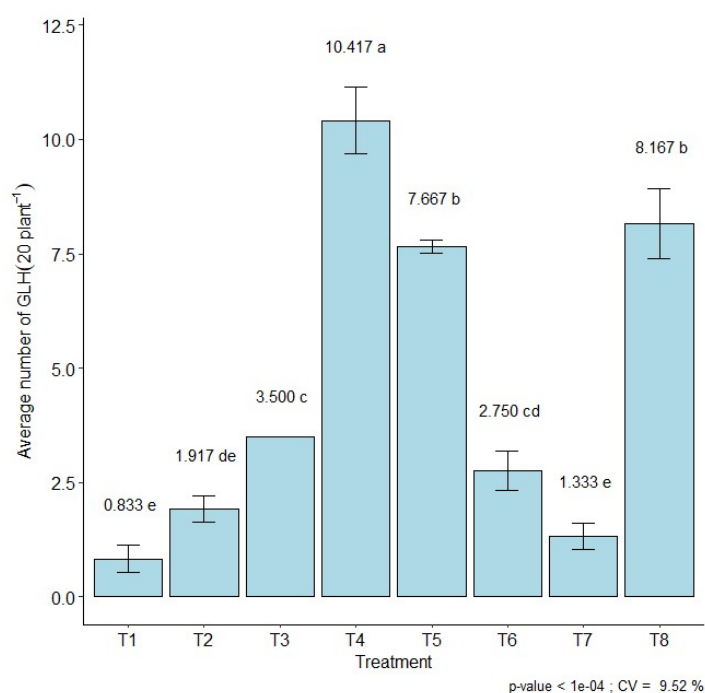


Figure 4: The average number of GLH found 20seedlings⁻¹ at different days after infestation

Note: T₁= Imidacloprid 70 WG; T₂=Curtap 50 SP; T₃=Isoprocarb 50 WP; T₄=Untreated Control; T₅=Tetrataniprole 20Sc; T₆=Cyantraniliprole 40 SC; T₇= thiamethoxam 25 WG; T₈=Buprofezin 40 EC.

3.5. Effects of insecticidal seed treatment on insects and natural enemy catches through sweeping at seedbed

The number of the rice whorl maggot (RWM) was observed significantly difference among different insecticides treatment (Table 3). The maximum number of the RWM was observed at untreated control (100.12) and curtap (94.56) treated seedbed, while the minimum was found at thiamethoxam (15.55) and buprofezin (25.39) treated seedbed. The maximum number of brown plant hopper (BPH) was observed at untreated control (7.12), while the minimum number of BPH was observed at thiamethoxam (0.92), imidacloprid (1.13) and buprofezin (2.05) treated seedbed (Table 3). The number of zigzag leafhopper (ZZLH) observed maximum at untreated control (2.83), isoprocarb (2.62) and curtap (2.76) treated seedbed and which was statistically identical, the minimum number of ZZLH was observed at imidacloprid (1.13), thiamethaxm (0.65) and buprofezin (1.28) treated seedbeds. The population of green leaf hopper (GLH) was observed maximum at untreated control (2.80) which was statistically identical with curtap (2.73) and isoprocarb (2.58) treated seedbeds (Table 3). Furthermore, the minimum number of GLH was observed at thiamethaxam (0.61) and imidacloprid (0.61) treated seedbeds. The percentage of GLH reduction over control varied significantly among the treated insecticides seedbed. As compared to untreated control, both imidacloprid (78.21%) and thiamethoxam (78.12%) exhibited highest reduction of the GLH infestation.

In addition, the number of natural enemies exhibited statistically significant difference except the parasitic wasps (PW) caught among different treated seedbeds. The populations of green mirid bug (GMB) was caught maximum at untreated control (30.67), followed by isoprocarb (21.22) and curtap (11.40), respectively. Furthermore, the minimum number of GMB was exhibited at tetrataniliprole (1.82) and buprofezin (1.58) treated seedbed. The population of spiders was caught maximum at untreated control (2.01) and imidacloprid (1.71) treated seedbed and which were statistically identical (Table 3).

Table 3: Average number of insects and natural enemies catches through weekly basis sweeping under seedbed from 1st week after sowing (WAS) to 8 WAS

Treatment	Rice Harmful Insects				Reduction (%) of GLH over control	Natural enemies		
	RWM	BPH	ZZLH	GLH		GMB	PW	SPD
T1=Imidacloprid 70 WG	39.99 c	1.13 de	1.13 cd	0.61 d	78.21 a	6.96 d	2.05	1.71 a
T2=Curtap 50 SP	94.56 a	4.53 b	2.76 a	2.58 a	7.45 d	11.40 c	4.77	0.76 b
T3=Isoprocarb 50 WP	80.93 b	4.68 b	2.62 ab	2.73 a	2.41 d	21.22 b	4.11	0.48 b
T4=Untreated Control	100.12 a	7.12 a	2.83 a	2.80 a	0.00 d	30.67 a	4.40	2.01 a
T5=Tetratanipole 20Sc	33.04 cd	2.98 bc	2.05 abc	1.02 c	63.00 b	1.82 e	3.52	0.52 b
T6=Cyantraniliprole 40 SC	33.93 cd	2.76 cd	1.70 bc	1.01 c	64.02 b	4.83 de	3.63	0.59 b
T7=Thiamethoxam 25 WG	15.55 e	0.92 e	0.65 d	0.61 d	78.12 a	3.21 de	3.92	0.78 b
T8=Buprofezin 40 EC	25.39 de	2.05 cde	1.28 cd	1.59 b	42.96 c	1.58 e	3.74	0.85 b
Levels of Significance	***	***	**	***	***	***	ns	***
Least Significance Difference	11.64	1.70	1.002	0.39	13.35	4.04	-	0.39
Coefficient of Variation (%)	12.70	30.05	30.81	14.02	18.35	22.83	-	23.68

Note, RWM: Rice whorl maggot; BPH: Brown planthopper; ZZLH: Zigzag headed leafhopper; GLH: Green leaf hopper; GMB: Green mirid bug; PW: Parasitic wasp; SPD: Spider

4. Discussion:

This current study assessed the effects of various insecticidal seed treatments on rice seed germination, the growth of seedlings, the percentage of seedlings damaged by green leafhoppers (GLH). Additionally, the dynamics of GLH populations, and the prevalence of harmful insects and their natural enemies in the rice seedbeds.

4.1. Effects on seed germination and seedling growth

The percentage of seed germination was varied significantly among treatments, all chemical insecticides treatment showed more than 86% seed germination (Table 2), indicated their no adverse effects on seed germination except curtap (63.33%). Under curtap treatment, reduced percentage of germination may be imposed to its phytotoxic effects on seed metabolism, especially by interfering with enzymatic activities engaged in seed germination (Roy et al., 2025b). On the other-hand, thiamethoxam and imidacloprid insecticides maintained comparatively higher germination rate (>86.33%), indicating improved seed vigor and less phytotoxic impacts. An investigation by (Afifi et al., 2015) and documented that maize seeds treated with thiamethoxam increased the production of gibberellic acid synthesis and decreased synthesis of abscisic acid, which influences germination patterns and also increased germination percentage in presence of weeds. Similar findings were documented by (Ding et al., 2018; Lanka et al., 2017; Xi et al., 2022) who reported that neonicotinoids insecticides such as imidacloprid, thiamethoxam and chlorantraniliprole were found to improve germination of seed, shoot and root length and vigor index in rice by modifying hormonal network which responsible for stress tolerance. The tested insecticides had a considerable impact on seedling vigor, as determined by the length of root and shoot. Longest root found by treating with imidacloprid (5.15 cm) and curtap (5.06 cm) while longest shoots found by treating with imidacloprid (3.44 cm), isoprocarb (3.62 cm) and untreated control (3.56 cm), indicating their possible growth promoting effects. Neonicotinoids may cause systemic physiological stimulation in plants at low concentrations which increased plant height, fresh mass of shoot, bio-mass of root, area of leaf, and photosynthetic efficiency for maize (Ding et al., 2018; House et al., 2021), rice (Roy et al., 2025b; Xi et al., 2022) and cotton (Sehrish et al., 2024).

4.2. Seedlings damage and suppression of GLH populations

Both no-choice and choice methods tests verified that untreated control seedlings suffered the maximum damage (choice: 50.67%, no-choice: 94.67%) of seedlings, whereas imidacloprid and thiamethoxam showed superior protection from GLH with minimum damage (<7.00%) followed by cyantraniliprole (Table 2; Figure 1). These results demonstrate the potent systemic activity of neonicotinoids (imidacloprid and thiamethoxam), which protect rice seedlings from early hopper infestations through ingestion-mediated toxicity, supported by (Ding et al., 2018; Roy et al., 2025b; Sehrish et al., 2024). According to (House et al., 2021), cyantraniliprole was found to be effective as seed treatment against *Lissorhoptrus oryzophilus* populations through and ineffective against stem borer. Moreover, a very few amount of insecticide was required for seed treatment of rice but the results showed a significant reduction at low application rates (g or mL hectare⁻¹) utilized per unit area for the early stage protection. Thus can be reduced large scale application of insecticides for managing GLH populations and also helps to protects natural enemies and environment. Moreover, when the seedling had get systemically toxic or anti-feedent due to seed treatments, then the number of GLH availability on seedling were reduced (Figure 2, Figure 3, Figure 4 & Figure 5). The untreated

control plots consistently demonstrated the maximum number of GLH, while thiamethoxam and imidacloprid were maintained the minimum populations, throughout the observation period. These findings corroborate with earlier findings and exhibited that long-lasting suppression of sucking pests like GLH was provided by neonicotinoids, thereby helps to reducing survival and feeding, by interfering with nicotinic acetylcholine receptors (Kundoo et al., 2018; Roy et al., 2025b; Sehrish et al., 2024; Uragayala et al., 2015). The plant's defense mechanism are enhance by inducing secondary metabolites against insect-pests through pesticide seed coating (AB Ghaffar et al., 2011; Xi et al., 2022). Rice phloem which fed by GLH, responsible for changes the chemistry of diverse immune regulators, such as phenolic compounds and oxalic acid, perhaps resulting in resistance to GLH (Roy et al., 2025b; Xi et al., 2022). Moreover, oxalic acid is frequently found in plants, fungi, bacteria, and animals. It serves as essential roles in plants including defence mechanism against herbivory (Usha Rani & Jyothsna, 2010). Furthermore, oxalic acid is an effective inhibitor, which interferes and disrupts the sucking function of GLH ability to grow (AB Ghaffar et al., 2011; Xi et al., 2022). In addition, by binding to the gustatory receptor Gr23a oxalic acid effectively inhibits the feeding behavior of hopper and thus leading to adverse responses to seedling (Kang et al., 2023), and prohibit GLH to infest rice seedling (Figure 5).

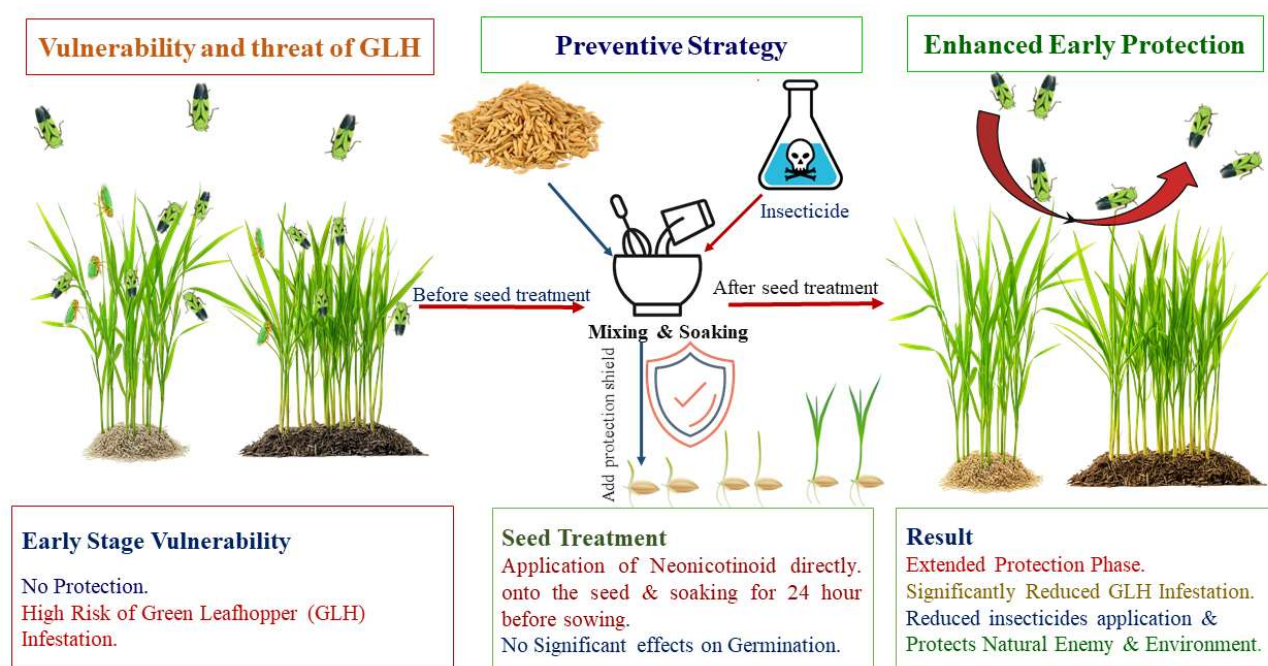


Figure 5: Seed treatment as a preventive measure against green leafhopper at early stage of rice

Our present findings confirmed that insecticide seed treatment particularly influenced the abundance of insect-pests when sweep net catches from seedbeds. The prevalence of GLH, rice whorl maggot (RWM), brown planthopper (BPH) and zigzag leafhopper were found higher at untreated control followed curtap and isoprocarb treatment. On the other hand, imidacloprid and thiamethoxam continuously showed minimum number of pest densities, confirming their effectiveness as protective agents. Seed treatment with thiamethoxam and imidacloprid had been shown to decrease thrips infestations and inhibit early season season insect colonization in maize (Ding et al., 2018). The insecticides of neonicotinoids are very systemic, particularly when employed as a seed dressing, due to their high water solubility, which makes it easy for both root systems and leaves to absorb and translocate (Roy et al., 2025b; Simon-Delso et al., 2015). These systemic

characteristics of neonicotinoids allow them to penetrate not only a sapling's roots and leaves but also surrounding soils of the roots zone, imparting them "excellent activity particularly against coleopteran (i.e. beetle species), lepidopteran (i.e. leafworms), dipteran (i.e. flies), and homopteran (i.e. aphids and leafhoppers pests)" (Ding et al., 2018; Kundoo et al., 2018; Sehrish et al., 2024; Uragayala et al., 2015). Because of these features, neonicotinoids are a wonderful complement to seeds and crops. Neonicotinoids seed coating provide immediate efficacy against harmful early-growth-stage insect-pests like hopper species while it takes three to six weeks for emerging seedlings to acquire enough to repel pests (Kundoo et al., 2018). In addition, as compared to untreated control, imidacloprid reduced 78.21% and thiamethoxam reduced 78.12% of GLH infestation (Table 3). Moreover, there were no visual "tungo" symptoms observed by naked eye in both greenhouse and seedbeds.

The abundance of natural enemies, especially spiders and green mirid bugs, were also significantly influenced by different seed treatments. The untreated control plots founds high abundance of natural enemies like green mirid bugs, spiders due to no insecticidal effects whereas isoprocarb plots and imidacloprid plots harbored the maximum number of spiders. Imidacloprid has been determined to have little adverse effect and to be safe for ladybird beetle and spiders (Chatterjee et al., 2009; Roy et al., 2025b; Wilson et al., 2021). However, the prevalence of natural enemies was decreased by neonicotinoids and broad-spectrum insecticides, which may interrupt the natural pest regulation (Roy et al., 2025b; Wilson et al., 2021; Zhang et al., 2016). The excessive use of insecticides might disrupt the equilibrium between predators and prey species in rice ecosystems (Roy et al., 2024; Wilson et al., 2021). Therefore, seed treatments can protect natural enemies from direct insecticide contact due to little quantity requirement and not direct applications, as opposed to foliar insecticide sprays. However, some insecticide's non-selective effects on natural enemies raise questions regarding ecological balance and long-term sustainability through seed treatment. Furthermore, spiders, green mirid bug and parasitic wasps are common natural enemies which are uses in worldwide as biological control programs, due to their high feeding potency, they are able to transition to a diet that contained very few insecticides in their prey. So, in order to protect natural enemies and environment, these insecticide might be used to protect rice at early stage against GLH.

5. Conclusion

The present study highlights that both neonicotinoid (thiamethoxam and imidacloprid) ensured high seed germination (86.33%) which indicated no adverse phytotoxic effects. Moreover, they were highly effective in reducing the GLH population and associated minimum seedling damage, thereby maintaining higher seedling vigor compared to other seed treatments. The effectiveness of these seed treatments was confirmed by reduced number of GLH and other harmful insect catches on treated seedbeds, emphasizing their potential to significantly reduce the need for early stage foliar insecticide applications. These findings underscore the need for neonicotinoid seed treatments as a rational pest management strategy, offering a path to reduce dependency on foliar sprays and while supporting the conservation of natural enemies and the environment. Therefore, further research should concentrate on this topic and need to investigate the impact of seed treating insecticide on natural enemy populations in broad aspects, which should judiciously combine selective insecticides, host plant resistance and biological control agents.

Author Contributions: “Conceptualization, methodology and investigation, T.K.R; formal analysis, T.K.R and A.S.; data curation, T.K.R., M.H.H., A.S., and J.Y.J.; writing—original draft preparation, T.K.R., M.H.H., A.S., and J.Y.J.; writing—review and editing, T.K.R., A.S., M.H.H., S.A., J.Y.J. M.M.K., A.N., M.N.B., A.U.M., S.T., and M.M.H.; supervision, T.K.R.; materials and equipment, T.K.R., M.H.H., M.N.B., and M.M.H. All authors have read and agreed to the published version of the manuscript”.

Funding: “This research received no external funding”.

Acknowledgement: “Authors are greatly thankful to the Scientific assistant, Chief Scientific Officer & Head, Entomology Division, Bangladesh Rice Research Institute (BRRI), Gazipur for the laboratory, greenhouse, assistance and equipment”.

Conflicts of Interest: “The authors declare no conflict of interest.”

Data availability statement: “All data analyzed during this study are included in this article. Moreover, data will make available on request”.

6. References

1. Ghaffar MAB, Pritchard J, Ford-Lloyd B (2011): Brown planthopper (*Nilaparvata lugens* Stål) feeding behaviour on rice germplasm as an indicator of resistance. PLoS ONE 6(7), e22137. <https://doi.org/10.1371/journal.pone.0022137>
2. Afifi M, Lee E, Lukens L, Swanton C (2015): Maize (*Zea mays*) seeds can detect above-ground weeds; thiamethoxam alters the view. Pest Management Science 71(9), 1335–1345. <https://doi.org/10.1002/ps.3936>
3. Alford A, Krupke CH (2017): Translocation of the neonicotinoid seed treatment clothianidin in maize. PLoS ONE 12(3), e0173836. <https://doi.org/10.1371/journal.pone.0173836>
4. Bari MN, Haque SS, Nowrin F, Ali MP, Kabir MMM, Akter S, Roy TK, Afrin S (2025): Eco-engineering for managing insect pests in rice fields. Bangladesh Rice Journal 27(1), 49–60. <https://doi.org/10.3329/brj.v27i1.77723>
5. Buchholz A, Nauen R (2002): Translocation and translaminar bioavailability of two neonicotinoid insecticides after foliar application to cabbage and cotton. Pest Management Science 58(1), 10–16. <https://doi.org/10.1002/ps.401>
6. Chatterjee S, Isaia M, Venturino E (2009): Spiders as biological controllers in the agroecosystem. Journal of Theoretical Biology 258(3), 352–362. <https://doi.org/10.1016/j.jtbi.2008.11.029>
7. Ding J, Li H, Zhang Z, Lin J, Liu F, Mu W (2018): Thiamethoxam, clothianidin and imidacloprid seed treatments effectively control thrips on corn under field conditions. Journal of Insect Science 18(6). <https://doi.org/10.1093/jisesa/iey128>
8. House MA, Swanton CJ, Lukens LN (2021): The neonicotinoid insecticide thiamethoxam enhances expression of stress-response genes in *Zea mays* in an environmentally specific pattern. Genome 64(5), 567–579. <https://doi.org/10.1139/gen-2020-0110>
9. IRRI (International Rice Research Institute) (2013): *Standard Evaluation System for Rice*. 5th ed. IRRI, Los Baños, Philippines.
10. Islam Z, Catling D (2012): *Rice Pests of Bangladesh: Their Ecology and Management*. University Press Limited.
11. Jeschke P, Nauen R, Schindler M, Elbert A (2011): Overview of the status and global strategy for neonicotinoids. Journal of Agricultural and Food Chemistry 59(7), 2897–2908. <https://doi.org/10.1021/jf101303g>

12. Kabir MMM, Hossain MA, Ali MP, Bari MN, Haque SS, Hossain ST, Datta J (2023): Management of insect pests in rice seedbeds using a novel rectangular hand net (RHN) for insecticide-free seedlings. *International Journal of Business, Social and Scientific Research* **11**(1), 19–20. <https://doi.org/10.55706/ijbssr11104>
13. Kang K, Zhang M, Yue L, Chen W, Dai Y, Lin K, Liu K, Lv J, Guan Z, Xiao S, Zhang W (2023): Oxalic acid inhibits feeding behavior of the brown planthopper via binding to gustatory receptor Gr23a. *Cells* **12**(5), 771. <https://doi.org/10.3390/cells12050771>
14. Kundoo AA, Dar SA, Mushtaq M, Bashir Z, Dar MS, Gul S, Ali MT, Gulzar S (2018): Role of neonicotinoids in insect pest management: A review. *Journal of Entomology and Zoology Studies* **6**(1), 333–339.
15. Lanka SK, Senthil-Nathan S, Blouin DJ, Stout MJ (2017): Impact of thiamethoxam seed treatment on growth and yield of rice (*Oryza sativa*). *Journal of Economic Entomology* **110**(2), 479–486. <https://doi.org/10.1093/jee/tox043>
16. Morshed MN, Mamun MA Al, Nihad SAI, Rahman MM, Sultana N, Rahman MM (2023): Effect of weather variables on seasonal abundance of rice insects in the southeast coastal region of Bangladesh. *Journal of Agriculture and Food Research* **11**, 100513. <https://doi.org/10.1016/j.jafr.2023.100513>
17. Roy TK, Akter S, Kabir MMM, Tonmoy SMMS, Sharmin S, Riad MA, Rana MM, Biswas MA, Uddin ABMA, Sannal A (2025a): Strategic management of rice stem borer: Evaluating tobacco-based alternatives to reducing insecticide dependency. *Scientia Agriculturae Bohemica* **56**(3), 1–13. <https://doi.org/10.7160/sab.2025.560313>
18. Roy TK, Kabir MMM, Akter S, Nayeem A, Alam Z, Hasan MR, Bari MN, Sannal A (2024): Seasonal variations of insect abundance: Correlating growth stage-specific metrics with weather patterns in Rangpur Region, Bangladesh. *Heliyon* **10**(18), e38121. <https://doi.org/10.1016/j.heliyon.2024.e38121>
19. Roy TK, Sannal A, Hena MH, Joaty JY, Biswas MA (2025b): Early-stage management of brown planthopper (*Nilaparvata lugens*) through seed treatment: A preventive strategy in rice cultivation. SSRN. <https://doi.org/10.2139/ssrn.5451722>
20. Sehrish A, Parajulee M, Vyavhare S, Coldren C, Laza H, Simpson CR (2024): Effects of neonicotinoid seed treatments on cotton seedling physiology, nutrition and growth. *Agronomy* **14**(4), 799. <https://doi.org/10.3390/agronomy14040799>
21. Shultana R, Ali Tan KZ, Rana M, Roy TK, Naher UA, Ibne Baki MZ, Khan MAI, Akter R, Paul PL, Shupta SA, Islam MS, Hasan AK (2025): Exploring the impact of agricultural pesticides on soil microbes: A comprehensive review. *Egyptian Journal of Soil Science* **65**(3). <https://doi.org/10.21608/ejss.2025.371094.2081>
22. Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke CH, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, et al. (2015): Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research* **22**(1), 5–34. <https://doi.org/10.1007/s11356-014-3470-y>
23. Tejaswi A, Jarpla M, Bandhavi HL, Prasanna M (2024): Evaluating the bio-efficacy of pesticide combinations on natural enemies in rice cultivation. *Journal of Advances in Biology & Biotechnology* **27**(10), 951–957. <https://doi.org/10.9734/jabb/2024/v27i101517>

24. Uragayala S, Verma V, Natarajan E, Velamuri PS, Kamaraju R (2015): Adulticidal and larvicidal efficacy of three neonicotinoids against insecticide-susceptible and resistant mosquito strains. *Indian Journal of Medical Research* **142**(Suppl. 1), S64–S70. <https://doi.org/10.4103/0971-5916.176624>
25. Usha Rani P, Jyothsna Y (2010): Biochemical and enzymatic changes in rice plants as a mechanism of defense. *Acta Physiologiae Plantarum* **32**(4), 695–701. <https://doi.org/10.1007/s11738-009-0449-2>
26. Wilson BE, Villegas JM, Way MO, Pearson RA, Stout MJ (2021): Cyantraniliprole: A new insecticidal seed treatment for U.S. rice. *Crop Protection* **140**, 105410. <https://doi.org/10.1016/j.cropro.2020.105410>
27. Xi C, Ahmad S, Yu J, Zhang J, Chen Y, Zhang G, Zhu H, Ge L, Yu X, Shu Z (2022): Seed coating with triflumezopyrim induces the rice plant's defense and inhibits the brown planthopper's feeding behavior. *Agronomy* **12**(5), 1202. <https://doi.org/10.3390/agronomy12051202>
28. Zhang P, Zhang X, Zhao Y, Wei Y, Mu W, Liu F (2016): Effects of imidacloprid and clothianidin seed treatments on wheat aphids and their natural enemies on winter wheat. *Pest Management Science* **72**(6), 1141–1149. <https://doi.org/10.1002/ps.4090>

Copyright: © 2026 by the authors. License SAB, Prague, Czechia. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).