

## Validation of plant-based Indigenous Traditional Knowledge (ITK) for management of *Sitophilus oryzae* in traditional storage structures

Surajit Kalita<sup>a,\*</sup>, Lakshmi Kanta Hazarika<sup>b</sup> & Junmoni Gayon<sup>b</sup>

<sup>a</sup>Directorate of Research (Agri), <sup>b</sup>Department of Entomology, Assam Agricultural University, Jorhat 785 013, Assam

\*E-mail: surjit\_kalita@yahoo.com

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Indigenous Traditional Knowledge (ITK) based on indigenous resources have been getting attention internationally and hence, scientific validation of the potential ITKs could help in the generation of innovative crop production and protection technologies in the near future. The experiment to validate Indigenous Traditional Knowledge (ITK) for management of storage insect pests was conducted at farmers' storage godown in Silpota and Saljhora village of Chirang district of Assam, and revealed that the application of *Polygonum hydropiper* dry leaf powder @ 10 g/kg of seed can effectively control the damage of rice seed kept in traditional storage structures caused by *Sitophilus oryzae* recording 55.36%, 44.57% and 44.80% seed damage reduction over pre-treatment count (PrTC) on top (0-15 cm), mid (15-30 cm) and lower (> 30 cm) layer, respectively as against an increase in seed damage ranging between 201.62-221.60% over PrTC at 90 days after treatment in the control. Application of dried whole plant of *P. hydropiper* @ 4.0 cm thick layer over the stored seed showed little impact on reduction in incidence of *S. oryzae* recording 100.93%, 116.28% and 111.67% increase in seed damage over PrTC in upper (0-15 cm), mid (15-30 cm) layer and lower (>30 cm deep) layer, respectively; suggesting effective release of plant bioactive compounds with sufficient and enhanced contact points for biological activity.

**Keywords:** Indigenous Traditional Knowledge (ITK), *Polygonum hydropiper*, Rice, *Sitophilus oryzae*, Seed damage, Traditional storage structure

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The evolution of pest management in rice has been inherited towards "Integrated pest management era" through the use of Indigenous Traditional Knowledges (ITKs) across different farming communities in India. Indian farming communities act as a vast repository of such traditional and technological knowledge pertaining to agriculture since time immemorial and are transmitted from one society to another in the form of social attitudes, beliefs, principles, and practices. Indigenous knowledge gets updated or modified from historical experiences and get stored in people's memory and expressed as stories, folklore, myths, beliefs, rituals, agricultural practices, etc. The greatest advantage of such traditional knowledge is lesser or no cost involvement apart from the use of local and easily available resources with limited environmental hazards. In severely degraded environments, ITKs could be the prime resources to extract mutual benefit to human societies by converting them into economically viable one through suitable

scientific validation and alterations. Exploration and documentation of ITKs had also indicated their suitability for suppressing several important insect pests<sup>1</sup>.

Rice being a dominant crop, Assamese farmers of different ethnic community has developed traditional knowledge practices based on their culture, tradition, and belief, which passed verbally from one generation to the next without having authenticated written documents except a few in the form of "*Dakar Prabasan*"<sup>1-3</sup>. Due to ignorance and apathy of the new generation towards traditional knowledge along with their weaknesses towards the modern technologies, a few have already been lost in time. It is therefore necessary to collect these ITKs available across the farming societies, analyse them critically to understand the science behind and disseminate the knowledge for their future use in reducing the pest menace<sup>1</sup>. Placing a layer of rice husk at the top of the stored bin, keeping of rice seeds without cleaning, application of a layer of dry neem leaves over the stored rice seeds, application of a layer of dry

\*Corresponding author

*Polygonum hydropiper* plants, and keeping of outenga, *Dellinia indica* cut into halves are some of the commonly practiced ITKs against storage insect pests<sup>4,5</sup>.

*Polygonum hydropiper* (Family - Polygonaceae) is a weed commonly grown in soil of low-lying areas containing high organic carbon. Flavonoids are the major group of phytochemicals present on *P. hydropiper* along with sesquiterpenes, sesquiterpenoids, and phenylpropanoids, which show pharmacological activities including antioxidant, antibacterial, antifungal, antihelminth, antifeedant, cytotoxicity, etc.<sup>6,7</sup> along with strong insecticidal properties<sup>8-13</sup>. On the other hand, rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) is cosmopolitan in nature amongst different storage insect pests, causing significant damage alone of stored cereals including rice, maize, wheat, barley, etc. The rice weevil causes heavy damage of more than 80% seed damage under protracted storage condition as compared to 10 - 65% seed damage under moderate storage<sup>14</sup>. Hence, the present investigation aims at validation of the ITK suggesting use of *P. hydropiper* dry leaves in storage structures against *S. oryzae*.

### Materials and Methods

The experiment to validate the ITK suggesting use of *P. hydropiper* dry leaves against *S. oryzae* was carried out in farmer's houses on a traditional

bamboo-based storage structure/bins (Make: Bamboo, Capacity: 100 kg) called "duli" (in Assamese) at Silpota (26.52°N, 90.51°E and 51.9 meter above MSL) and Saljhora (26.51°N, 90.49°E and 61.7 meter above MSL) villages of Chirang district of Assam during 2017-2019. The locality has been characterized by a hot (temperatures reaching a maximum of 35-38°C during summer and a minimum 6-8°C during winter) and humid (Average annual relative humidity ranges 75-80%) climate, supporting a higher insect pest infestation during the storage of seeds. The locally prepared bamboo storage bins were made up of untreated, fresh and matured *Jati* bamboos (*Bambusa tulda*), smeared with a paste made up of fresh cowdung and clay soil (1:1 ratio) with the addition of water and dried the bamboo bin under sun before storage of paddy seed (Fig. 1). Naturally grown *P. hydropiper* plants were collected locally, shade dried, and stored in polythene packets for use in the experimentation; otherwise, shade dried *P. hydropiper* leaves were powdered and stored in airtight containers under refrigeration for further use. At first, rice grains (Variety- Ranjit, a popular rice variety of Assam with medium long grain with moderate susceptibility to insect pest infestation) weighing around 100 kg was dried under sun and cooled under shade before placement on the bamboo storage structures. Each of the bamboo bins were placed over a bamboo platform 2 feet above the



Fig. 1 — Use of *Polygonum hydropiper* for management of *Sitophilus oryzae*. (a) Collection and drying of *P. hydropiper* plant parts, (b) Plant sample preparation for experimentation, (c) Traditional storage structure utilized in the experimentation, (d) Admixing of *P. hydropiper* leaf powders, (e) Application of dried *Polygonum hydropiper* plant parts over the paddy seeds, (f) Mixing of dried *Polygonum hydropiper* leaf powder with the paddy seeds

ground. Alongside, mass cultured and mother progenies of the rice weevil, *S. oryzae* reared on paddy seed (Var. Ranjit) were collected from the Post Harvest Engineering Technology Laboratory, Assam Agricultural University, Jorhat and artificially inoculated @ 50 pairs of neonates of *S. oryzae* (Male: Female = 1:1 ratio) were released per replication and waited till minimum of 5.26-6.10% seed damage was observed as pre-treatment count (PrTC) across the treatment replications. Afterward, a layer of dry *P. hydropiper* plants at 1.0, 2.0, 3.0 and 4.0 cm thickness was spread over the infested seeds stored on the storage structure. On the other treatments, *P. hydropiper* leaf powders were admixed with the infested seeds @ 5 g/kg and 10 g/kg of seed separately. Each treatment was replicated 5 times with separate storage bin for each replication and data on seed damage (%) was recorded at different layers of stored rice seeds *i.e.*, top (0-15 cm), middle (15-30 cm) and lower (> 30 cm). A control with paddy seed (Var. Ranjit) was maintained alongside the treatments in separate storage bin to compare the bioefficacy of *P. hydropiper*. The experiment was conducted under ambient climatic condition (Temperature ranges: 28-37°C and Relative Humidity: more than 80% during experimentation) and the data at different time interval on seed damage was recorded by taking samples with the help of rice seed auger (Make: General) causing minimal disturbances. A pre-treatment count (PrTC) was also taken into consideration for estimating the period changes in seed damage over the initials and to estimate the changes in seed damage (%) over PrTC at periodic interval. Moreover, changes in seed damage (%) over the control were also calculated to rationalize the bioefficacy of *P. hydropiper* on *S. oryzae* in comparison farmers practices. In order to evaluate the effect of *P. hydropiper* on germination of rice seed, a representative sample from each of the treatment replications were collected at 90 days after treatment, grown on glass petri plates (Make: Borosil, Size: 10 cm diameter) as per standard procedure<sup>15</sup> and data on seed germination (%) was recorded at 7 days after sowing. Data so obtained were subjected to Analysis of Variance (ANOVA) under completely randomized block design as per the standard methods described by Gomez and Gomez<sup>16</sup>.

## Results

A concentration and time dependent reduction in seed damage on the upper (0-15 cm) layer of stored

rice was recorded due to application of *P. hydropiper* dry leaf layer and leaf powder (Table 1). The *P. hydropiper* @ 10 g/kg seed was the best recording the lowest seed damage of 5.68%, 5.80%, 6.00%, 6.40%, 7.28% and 8.60% at 7 days after treatment (DAT), 14 DAT, 21 DAT, 30 DAT, 60 DAT and 90 DAT, respectively as against 7.22%, 9.48%, 11.76%, 13.70%, 16.14% and 17.66% in the control. *Polygonum hydropiper* @ 5 g/kg recorded 5.84%, 6.0%, 6.80%, 8.14%, 9.60% and 11.40% seed damage at 7 DAT, 14 DAT, 21 DAT, 30 DAT, 60 DAT and 90 DAT, respectively. The leaf layer application @ 4.00 cm thick was the best in reducing seed damage by 19.11% and 35.45% over the control at 7DAT and 90 DAT, respectively in the upper (0-15 cm) layer of the seeds. While studying the middle (15-30 cm) layer of the rice on application dry *P. hydropiper* dry leaves and leaf powders (Table 1), it was found that *P. hydropiper* @ 10 g/kg seed resulted the highest of 19.44% reduction of seed damage over control at 7 DAT. The reduction in seed damage was found to vary between 38.84- 53.07% up to 90 DAT (Table 1). The treatment with *P. hydropiper* @ 1.0 cm thick layer was the least effective, recording only 5.83% seed damage reduction at 7 DAT, which eventually got increased to 16.76% reduction at 90 DAT in the middle (15-30 cm) layer as compared to the control. At the lower (>30cm deep) layer of the stored rice, application of *P. hydropiper* @ 10.0 g/kg proved to be the best treatment in reducing the seed damage 24.48% and 37.37, 44.73%, 50.99%, 55.36% and 51.10% over control (Table 1) at 7 DAT, 14 DAT, 21 DAT, 30 DAT, 60 DAT and 90 DAT, respectively. The treatment with *P. hydropiper* @ 5.0 g/kg recorded 50.27% reduction in seed damage over control at 90 DAT. *P. hydropiper* @ 4.0 cm recorded 11.70% seed damage as compared to 18.30% in the control at 90 DAT revealing 36.07% reduction in seed damage over the control (Table 1).

Application of *P. hydropiper* dry leaves showed a significant impact on seed damage caused by *S. oryzae* (Fig. 2-4) during the experimentation. There has been a reduction in seed damage over the control to a tune of -54.89%, -53.09% and -55.36% was recorded in the treatment with *P. hydropiper* leaf powder @ 10.0 g/kg of seed as against the lowest of -20.20%, -20.00% and -21.43% in the treatment with *P. hydropiper* @ 1.0 cm thick layer over the paddy seed on upper (0-15 cm) layer, middle (15-30 cm) layer and lower (>30 cm deep) layer, respectively at 90 DAT.

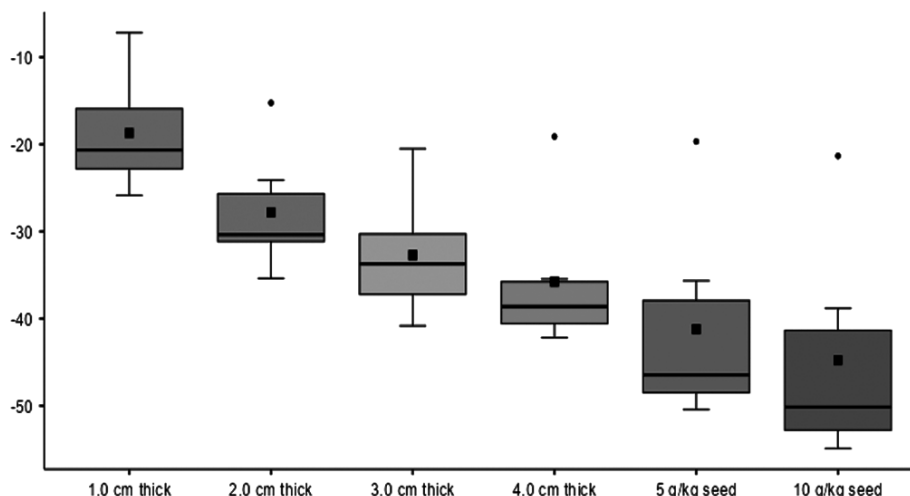


Fig. 2 — Effect of *P. hydropiper* on reduction in seed damage (%) over the control in upper (0-15 cm) layer of stored rice seed

Table 1 — Effect of *P. hydropiper* on seed damage (%) caused by *S. oryzae* (Pooled data)

Treatments	Seed damage (%) ± SEM						
	Upper (0-15 cm) layer						
	PrTC	7DAT	14DAT	21DAT	30DAT	60DAT	90DAT
1.0 cm thick	5.90±0.20	6.70±0.09	7.48±0.18	8.72±0.11	10.50±0.17	12.88±0.10	15.10±0.23
2.0 cm thick	5.62±0.28	6.12±0.17	6.60±0.10	7.60±0.19	9.40±0.24	11.24±0.18	13.40±0.18
3.0 cm thick	5.52±0.22	5.74±0.13	6.40±0.14	6.96±0.14	8.50±0.18	10.50±0.10	12.44±0.10
4.0 cm thick	5.70±0.16	5.84±0.07	6.00±0.10	6.80±0.16	8.14±0.26	9.60±0.18	11.40±0.29
5 g/kg seed	5.80±0.20	5.80±0.20	6.10±0.18	6.50±0.17	7.10±0.14	8.00±0.04	9.08±0.14
10 g/kg seed	5.60±0.18	5.68±0.13	5.80±0.22	6.00±0.10	6.40±0.11	7.28±0.17	8.60±0.13
Control	5.54±0.30	7.22±0.14	9.48±0.20	11.76±0.25	13.70±0.36	16.14±0.17	17.66±0.24
S.Ed. (±)	0.32	0.20	0.24	0.24	0.32	0.20	0.28
C.D. (0.05)	NS	0.40	0.49	0.49	0.65	0.42	0.57
C.D. (0.01)	NS	0.54	0.66	0.66	0.87	0.56	0.78
	Middle (15-30 cm) layer						
1.0 cm thick	5.90±0.14	6.78±0.13	7.70±0.32	8.82±0.28	9.40±0.19	12.96±0.19	14.90±0.15
2.0 cm thick	5.54±0.18	6.30±0.09	7.08±0.59	7.92±0.10	8.36±0.20	11.20±0.32	12.90±0.14
3.0 cm thick	5.36±0.12	6.00±0.12	6.82±0.32	7.60±0.11	7.80±0.14	10.40±0.16	11.92±0.23
4.0 cm thick	5.26±0.21	5.74±0.15	6.20±0.55	6.86±0.27	7.60±0.18	10.12±0.14	11.40±0.28
5 g/kg seed	5.58±0.11	5.96±0.16	6.28±0.34	6.64±0.09	7.00±0.20	8.50±0.16	9.20±0.23
10 g/kg seed	5.80±0.18	5.80±0.14	6.14±0.33	6.30±0.15	6.20±0.15	7.60±0.17	8.40±0.16
Control	5.94±0.16	7.20±0.21	10.04±0.61	11.48±0.11	14.88±0.54	16.20±0.23	17.90±0.19
S.Ed. (±)	0.23	0.21	0.29	0.25	0.25	0.28	0.29
C.D. (0.05)	NS	0.43	0.59	0.51	0.52	0.58	0.59
C.D. (0.01)	NS	0.58	0.80	0.69	0.70	0.79	0.80
	Lower (>30 cm deep) layer						
1.0 cm thick	6.00±0.16	7.22±0.13	8.18±0.14	9.30±0.15	10.90±0.13	13.20±0.19	14.90±0.19
2.0 cm thick	5.60±0.15	6.44±0.08	7.62±0.12	8.50±0.06	10.14±0.24	11.68±0.26	12.96±0.39
3.0 cm thick	5.60±0.13	6.12±0.24	7.00±0.20	7.90±0.26	9.10±0.20	10.74±0.22	12.00±0.38
4.0 cm thick	5.50±0.13	6.00±0.17	6.60±0.17	7.40±0.03	8.70±0.35	10.30±0.27	11.70±0.21
5 g/kg seed	5.60±0.24	5.70±0.12	6.20±0.15	6.60±0.13	7.20±0.17	8.20±0.16	9.10±0.22
10 g/kg seed	5.80±0.20	5.80±0.18	6.20±0.17	6.50±0.22	6.90±0.10	7.50±0.22	8.40±0.07
Control	6.10±0.19	7.68±0.17	9.90±0.24	11.76±0.32	14.08±0.37	16.80±0.14	18.30±0.39
S.Ed. (±)	0.25	0.23	0.25	0.27	0.35	0.30	0.41
C.D. (0.05)	NS	0.47	0.51	0.56	0.71	0.62	0.83
C.D. (0.01)	NS	0.63	0.69	0.76	0.95	0.84	1.12

- Data represented are the mean of 5 replications
- Sample size: 25 Nos.
- DAT: Days after treatment; PrTC: Pre Treatment counts

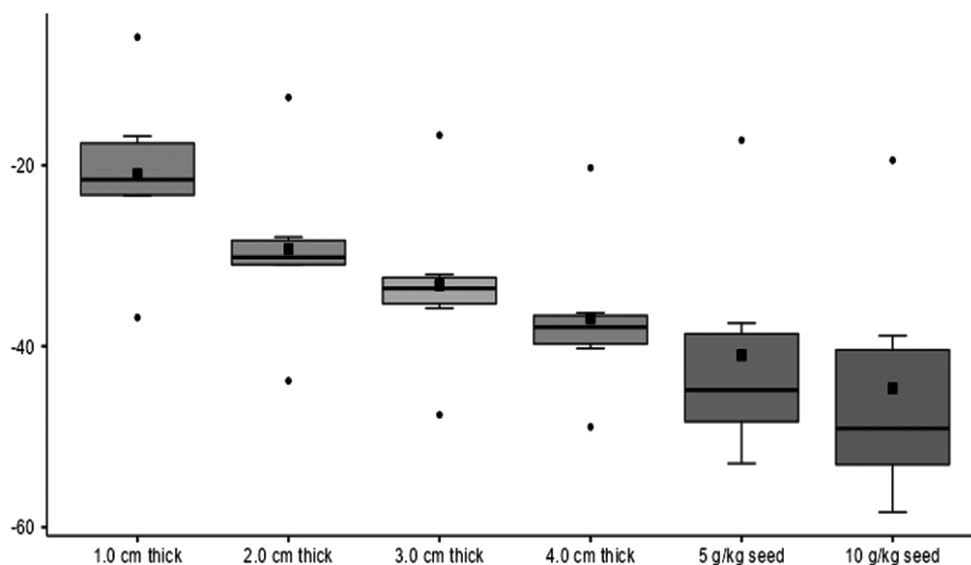


Fig. 3 — Effect of *P. hydropiper* on reduction in seed damage (%) over the control in middle (15-30 cm) layer of stored rice seed

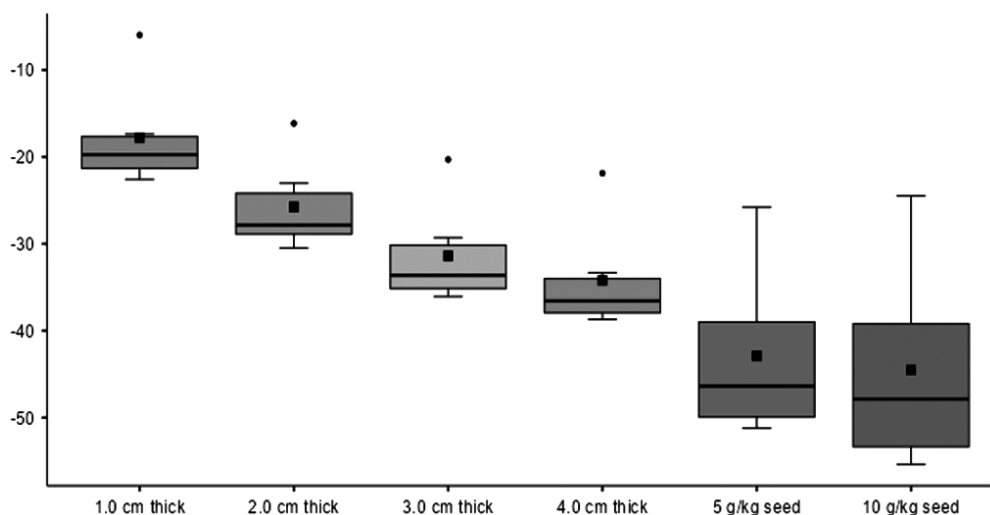


Fig. 4 — Effect of *P. hydropiper* on reduction in seed damage (%) over the control in lower (>30cm) layer of stored rice seed

It is interesting to note that the incidence of *S. oryzae* start resurging after 60 DAT, with slight increase in seed damage (%) could be recorded at 90 DAT. At 7 DAT, the reduction in seed damage (%) over the control was recorded to be the highest (-21.33%, -19.44% and 24.48%) in treatment with *P. hydropiper* leaf powder @ 10 g/kg seed in upper (0-15 cm) layer, middle (15-30 cm) layer and lower (>30 cm) layer, respectively as compared to the lowest of -7.20%, -8.83% and -5.99% in the treatment with 1.0 cm thick layer of dried *P. hydropiper* plant, respectively. Application of 4.0 cm thick layer of dried *P. hydropiper* plant cause reduction in the seed damage (%) in initial treatment hours upto 21 days recording -as high as -42.18%, -40.24% and -37.07%

reduction in seed damage in upper (0-15 cm) layer, middle (15-30 cm) layer and lower (>30 cm) layer, respectively, which is comparable with treatment with *P. hydropiper* leaf powder @ 5 g/kg seed recording -44.73%, -42.16 and -43.88% reduction in seed damage, respectively at 21 DAT.

While evaluating the effect of *P. hydropiper* on rice seed damage caused by *S. oryzae* over the pre-treatment count (PrTC) in the upper (0-15 cm) seed layer, it was found that *P. hydropiper* @ 10 g/kg of seed recorded no significant increase in seed damage at 7 DAT, which was found true for all the layers of stored rice seeds. The treatment with *P. hydropiper* @ 5 g/kg seed recorded no significant increase in seed damage on the upper (0-15 cm) layer; while 6.55%

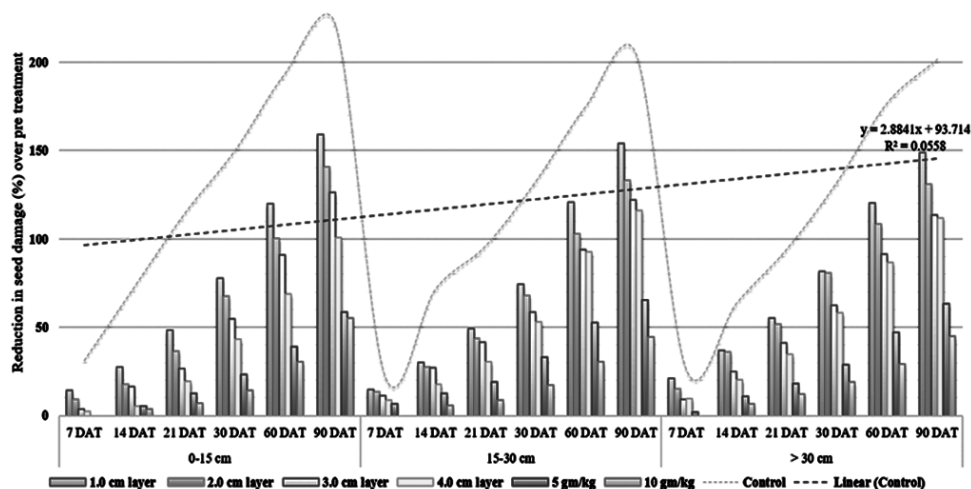


Fig. 5 — Effect of *Polygonum hydropiper* on reducing seed damage (%) over the pre-treatment count at different time interval

and 1.94% increase in seed damage was recorded over the PrTC in the mid (15-30 cm) and lower (>30 cm deep) layer, respectively. At 90 DAT, *P. hydropiper* @ 10 g/kg recorded 55.36%, 44.57% and 44.80% increase over PrTC in upper (0-15 cm), mid (15-30 cm) and lower (>30 cm deep) layer, respectively; while treatment with *P. hydropiper* @ 5 g/kg recorded 58.39%, 52.52% and 63.21%, respectively. Treatment with *P. hydropiper* @ 4.0 cm layer recorded 100.93%, 116.28% and 111.67% increase in seed damage over PrTC in upper (0-15 cm), mid (15-30 cm) layer and lower (>30 cm deep) layer, respectively suggesting a little effect of *P. hydropiper* dry plant layers in controlling *S. oryzae*, but the treatment showed some positive effects recording only 2.39% and 5.15% increase in seed damage over PrTC as early as 7 DAT and 14 DAT, respectively (Fig. 5).

The *P. hydropiper* treated seeds were also tested for possible effect on seed germination (%) and results were presented in (Fig. 6). Admixing of *P. hydropiper* leaves with rice seed revealed non-significant effect on seed germination, and the seed germination ranging between 90.80-91.60% across the treatments as compared to 91.40% in the control.

## Discussion

Although food security in terms of cereals has already been achieved in India, but minimization of post-harvest losses under storage condition, could be a viable and sustainable option for the food and nutritional security for the ever-growing populations of India. Post-harvest protection of agricultural produce can also contribute immensely towards



Fig. 6 — Effect of *Polygonum hydropiper* on seed germination (%) after 90 days of treatment

economic stability of the agricultural produce as well as resource conservation. Although, synthetic chemical pesticides such as organophosphates were widely been used for management of stored insect pests previously, however, seeing many negative consequences including residual toxicity, environmental pollution, and more often biomagnifications of persistent pesticides in the higher food chains, search for alternative storage insect pest management strategies gained a momentum in las decade<sup>17,18</sup>. Natural plant-based

products containing a range of bioactive chemicals including flavonoids, terpenoids, volatile oils, etc. are the good alternatives and substitute source of insect control under storage inducing strong effect as toxicant and reproductive inhibitors. Hundreds of bioactive compounds including vanicoside, ketopinonesinol, isorhamnetin, isalpinin, cardamomin, pinosylvin and  $\beta$ -sitosterol were isolated from the leaves of *P. hydro Piper*, which might play a major role in bringing toxicity against the insect<sup>19</sup>. The bicyclic sesquiterpenoid, polygodial found on the leaves are responsible for the pungent smell and taste<sup>7</sup>. Some of the biological compounds isolated from *P. hydro Piper* showed inhibition of tyrosinase activity, which might be responsible for the bioactivity against the storage pests. Saponins, a class of steroidal or triterpenoides found in the *P. hydro Piper* leaves showed diverse biological activities<sup>20</sup>, which might be responsible to bring biological activity against *S. oryzae*. The reduction in seed damage might be due to the presence of sulphated, methylated and glycosidal flavonoids like quercetin, isorhamnetin and rhamnazin that are naturally on the leaves of *P. hydro Piper*, which inhibits linoleic acid peroxidation and prevents generation of superoxide anion<sup>21,22</sup>. Altogether 5 flavonoids *viz.*, hyperin, rutin, quercetin, isorhamnetin and kaempferol along with volatile oil, tannins and other components with were isolated from *P. hydro Piper*<sup>23</sup>, which might be responsible for the anti-inflammatory, antioxidant, analgesic effects and most importantly, the insecticidal *Spilarctia oblique*, *Spodoptera litura*, *Oligonychus coffeae*, *Helicoverpa armigera*, *Aphis craccivora*, *Apion clavipes*, *Maruca vitrata* and *Omiodes indicata*<sup>10-14</sup>. Similarly, Keim *et al.*<sup>24</sup> has also reported that hydro Piperoside B, vanicode A and vanicode E isolated from *P. hydro Piper* also exhibited antioxidant activity. Several researchers had already shown the toxic effects of *P. hydro Piper* against storage insect pests, which corroborates our present investigation. Ayaz *et al.*<sup>25</sup> reported that butanol solvent extract of *P. hydro Piper* @ 500  $\mu$ g/ml recorded the highest of 88.90% and 93.33% mortality of *T. castaneum* and *Rhyzopertha dominica*, respectively. The insecticidal property exhibited by *P. hydro Piper* might be due to the presence of farnesol<sup>26</sup>, methyl palmitate and myristic acid<sup>27</sup>. There has been slight increase in seed damage (%) could be recorded at 90 DAT, which is due to degradation of plant bioactive molecules, loosing

sustained effectiveness against the target pests over the time period. The volatile oils including terpenoids and flavonoids present on *P. hydro Piper* exhibits insecticidal properties through disruption of cell membranes as well as impairing activities of acetylcholinesterase and glutathione-S-transferase in insects leading to death<sup>19</sup>. Moreover, there has been an increased bioefficacy recorded in the case of treatment with powdered plant leaf admixing as compared to treatments with dried whole plant parts, suggesting effective release of plant bioactive compounds with sufficient and enhanced contact points for biological activity<sup>28</sup>. Although varietal traits also play a role in minimizing the attach of insect pests, but the seed damage caused by *S. oryzae* in stored paddy seed was found less in traditional rice varieties as compared to the improved varieties, which might be because of morpho-chemical properties like grain size, hardness and shape in rice. Being a high yielding rice variety, *Ranjit* has been found to moderately susceptible to the infestation of *S. oryzae*<sup>15</sup>.

## Conclusion

Scientific validation of the potential ITKs have been getting attention internationally, which could help in the generation of innovative and sustainable crop production and protection technologies in the near future. Present investigation revealed effectiveness of *P. hydro Piper* leaf powder in reducing incidence of *S. oryzae* in traditional storage structures of Assam up to three months of storage period, which shall serve as a traditional knowledge-based sustainable and ecofriendly storage insect pest management, ensuring global food security in the near future.

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## Author Contributions

Investigation, methodology, data recording, analysis, and writing (SK); Conceptualization, research supervision, and reviewing (LKH); logistic support, methodology, writing (JG)

### Conflict of Interest

The authors declare that they have no conflict of interest in the manuscript's content and study undertaken.

### Ethical Statement

This article does not involve any studies on humans and animals.

### Data Availability

The data that support the findings of this study are available with the corresponding author, which could be obtained upon reasonable request.

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