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Scientific validation of two indigenous plants used for traditional storage pest management in Manipur

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Goniothalamus sesquipedalis and *Isodon ternifolius* are two well-documented ethnobotanically important plants used in Manipur by different tribes. It is also often used together as a traditional fumigant by burning and smoking by hanging in traditional granaries to reduce the insect pest infestation. This study scientifically validates the use of these two plants in different proportions to manage storage pests. The suppressive effect of plant powders on population increase through the inhibition of off spring was assessed. The study and evaluation focused on defined combined ratios of 50:50, 60:40, 70:30, and 80:20 of *Goniothalamus* and *Isodon*. The lowest GI_{50} of 2.92 mg/g was observed in *G. sesquipedalis* treated rice grains and exhibited the best suppression of the population growth of rice weevil among all the treatments evaluated. The subsequent best treatment was *I. ternifolius* with a GI_{50} of 3.00 mg/g and very similar to *G. sesquipedalis* in growth inhibition capability. Among treatment combinations, *Isodon* and *Goniothalamus* combined in a ratio of 60:40 displayed good grain protection from rice weevil damage with a low GI_{50} of 5.42 mg/g. The highest GI_{50} of 13.60 mg/g was shown by the combination of *Isodon* and *Goniothalamus* in the ratio of 70:30. The statistical tests of treatment means were highly significant at 1% level of significance, as the p-values in all cases are less than 0.01 except for one (80:20) where it was non-significant. The viability of seeds treated with *G. sesquipedalis* and *I. ternifolius* were found to be both enhanced. However, the seeds treated with a combination of *G. sesquipedalis* and *I. ternifolius* failed to germinate ultimately.

Keywords: *Goniothalamus*, *Isodon*, ITK validation, Manipur, Rice weevil, Storage pest

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India's North-East Region (NER) is a treasure house of flora, fauna, and fungi and a vast reserve of rich cultural heritage and traditions, including ancestral wisdom and knowledge about using the abundant natural bio-resources in daily life. The ITKs, also known as traditional wisdom, are based on indigenous practices passed on from generation to generation and reflected the vast experiences of a local indigenous population. ITK serves as the cornerstone for local decision-making in crop management including disease and pest control with soil management, preparation of local cuisine, health care, and other natural resource management (NRM), and the basis of different activities in tribal societies. Agriculture in the NER is considered complex, diverse, and risk-prone[1]. Farming practices and the agricultural ecosystem is fundamentally distinct from other regions of the country, e.g., shifting cultivation practised predominantly in the hill states. Agriculture in the region has many diversities and complexities in every aspect, giving uniqueness to it.

Although traditional knowledge is a valuable wealth of the people, it is common knowledge that it is slowly fading into oblivion with increasing modernity, rising population, and industrialization of everyday lifestyle[2]. Over time many indigenous medicinal plant species have become extinct before their documentation and validation. There is a lack of standardization concerning raw materials, production methods, and quality control of the finished product[3]. Until the late 1980s, the developed nations paid little attention to ITK, and underdeveloped countries received little help to preserve, collect, and systemize this knowledge. However, this mindset is slowly undergoing a sea change, and there is increasing healthy respect for the indigenous peoples and their knowledge.

The prevention and reduction of post-harvest food losses are critical for ensuring global food security in

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a sustainable manner[4]. Protecting grains against insect pests is a vital aspect of post-harvest food protection. The primary focus of global agricultural policy has been on increasing agricultural output and productivity through pest management. However, there has been insufficient attention given to the issue of post-harvest losses. Gustavsson *et al*. (2011) found that around one-third of the food produced worldwide, equivalent to around 1.3 billion tonnes valued at nearly $1 trillion, is wasted annually as a result of post-harvest losses[5]. Rice weevil, *Sitophilus oryzae,* is one of the most important pests of stored products globally and is the dominant storage pest of food grains and pulses under the highly humid climate of NE India[6]. Feeding by adults and grubs of rice weevil can reduce grain weight by as much as 75% and decreases the aesthetic and nutritional value of the grains[7].

Goniothalamus sesquipedalis and *Isodon ternifolius,* as shown in Figure 1, are two ethnobotanically important plants used by the farmers in Manipur by burning and smoking in traditional granaries to reduce pests and diseases incidence[8]. These plant components are combined with seeds, suspended in storage rooms, or incinerated to produce smoke prior to stocking a fresh harvest[9]. The Rongmei tribe of Manipur also uses the two plants together in multiple ways. The Rongmeis also drink a mixture of ashes (from the burnt leaves) with water to cure colic pain and stomachache in both children and adults. Konsam *et al.* (2015) also reported the use of *G. sesquipedalis* along with *Isodon ternifolius* in Manipur, one of the states of North-East India, in pre-and post-natal care as well as in traditional fumigation[10]. These plants have been extensively studied and are widely recognized for their usage in the traditional medicine and cultural practices of Northeast Indian communities.

Fig. 1 — Two ethnobotanically important plants used for storage pest management in Manipur

The absence of proper documentation and validation of informal knowledge systems, combined with the introduction of contemporary technology and agrochemicals, has resulted in a significant gap between traditional farming methods and technologically advanced agricultural practices, particularly in the area of plant protection. Ethnobotanical crop protection refers to the deliberate and organised application of plant extracts for the purpose of managing insect pests. These botanical pesticides have a wide range of qualities, such as toxicity, repellency, antifeedant effects, and insect development inhibition, which are effective against pests that affect major crops in agriculture. They are more affordable and readily accessible since they exist naturally in sufficient quantities, with minimal impact on the environment and human health[11].

Few scientific documents validate the grain protective ability against insect pests of certain indigenous plants used traditionally by farmers. Although the traditional knowledge of the use of these plants has been documented, as previously mentioned, there is no scientific validation done on the efficacy of these two plants, either alone or in combination, in protecting seed grains against storage insect pests. In the present global scenario, where the firm emphasis is on sustainable crop production and crop protection systems, traditional empirical knowledge could serve as the foundations of modern solutions to long-term problems by combining traditional wisdom with scientific technology. In India, only 30% of total food grains produced are held at the government level, and the remaining 70% are handled and stored at the farmers' level[12]. From this statistic alone, the tremendous role played by ITK using plants and their parts in post-harvest storage pest management in India can be well understood and acknowledged.

In the present study, an attempt has been made to standardize and scientifically validate the use of these two ethnobotanically important plants in different proportions to manage storage pests.

Materials and Methods

The entire work was done at ICARNEH Region, Manipur Centre, Lamphelpat, Imphal-795004, Manipur, between 2018 to 2020. The leaves of *G. sesquipedalis* and *I.* sold in small bundles were bought from the local markets and correctly verified by scientist for their respective identities. Subsequently, the material was dried in the shade and pulverised into fine powders using a high-quality industrial mixer and grinder. A study was designed to investigate the effectiveness of these native flora in inhibiting the population expansion of the rice weevil, *S. oryzae* using the same procedure as previously described by Ningombam *et al.,* (2021)[6]. Each powder, which had been dried in the shade and finely powdered, was combined with rice grain in varying amounts. Observations were then made every week to track the rise of the insect population. A total of seven treatment dosages of crude plant powder, ranging from 0.5 to 2.5 g, were examined. The experiment in the laboratory was conducted using a completely randomised block design with three replications. A replicate consisted of 50 g of rice grains mixed with treatment dose, as depicted in Figure 2. Observations were kept on a weekly basis. The population expansion was observed by tallying the offspring produced by the paired insects. Farmers reserve a portion of their produced foodgrain crops as seeds for the following season, and a period of 12 weeks was dedicated to making observations. The plant powders from both species were combined in various defined ratios, including 50:50, 60:40, 70:30, and 80:20. The same procedure was repeated for the combination of *G. sesquipedalis* and *I. ternifolius* plant powder. Untreated rice grains were used as a control in all treatments.

The growth inhibition in percentage was calculated using the formula given by Tapondjou *et al.* (2002)[13]:

Fig. 2 — Plant powder mixed with rice grain

GI %= (number of insects in control-number of insects in treatment)/number of insects in control × 100

Data analysis

Data were analyzed using the F-test by one-way analysis of variance, with means compared at 0.05 probability level ($p \leq 0.05$) A regression analysis was conducted in Microsoft Excel to examine the relationship between the logarithm of the dose and growth suppressed (%) in the treated grains. Regression equations were generated for each treatment, and the GI_{50} was determined. GI_{50} represents the dosage at which the growth of 50% of the insect population was suppressed. The population growth-inhibiting potential of the rice variety RC Maniphou-12 was assessed using plain white rice grains. This study testing the grain protective ability against insect pests was conducted on regular rice grains of rice variety RC Maniphou-12.

Effect on seed germination

Furthermore, research was conducted on the impact of these plants on the health of rice seeds of the same variety using the procedure previously described[6]. For a period of three months, rice seeds were treated at the same dosages as the grains with finely powdered powders. The treated rice seeds were then subjected to germination experiments using germination paper and petri-dish techniques. For each treatment, ten seeds were randomly selected in triplicates, and a germination test was performed. Five days after test was the initial count of germinated seeds, and seven days later was the last count.

Results and Discussion

For most cereals, the recommended moisture content range is 12-13%, as suggested by Lipinski *et al.* (2013)[14]. The rice grains used in this study had a mean moisture content of 12.65% when measured using a Grain Analyzer. The study results are reproduced in Table 1, where the effectiveness of each treatment was compared through their respective $G1_{50}$. Most of the treatments showed good ability to suppress insect population growth. The GI_{50} spanned from 2.92 to 13.60 mg/g. Out of six treatments evaluated, both singly and combined in fixed ratios, four treatments showed very high property of suppressing the growth of insect population while the remaining two treatments were not so good in comparison.

The lowest GI_{50} of 2.92 mg/g was observed in *G. sesquipedalis* treated rice grains and exhibited the best suppression of the population growth of rice weevil among all the treatments evaluated. The subsequent best treatment was *I. ternifolius* with a GI_{50} of 3.00 mg/g and very similar to *G. sesquipedalis* in growth inhibition capability. Among treatment combinations, *Isodon* and *Goniothalamus* combined in a ratio of 60:40 displayed adequate grain protection from rice weevil damage with a low GI_{50} of 5.64 mg/g. *Isodon* and *Goniothalamus* combined in 80:20 ratios also showed a low GI_{50} of 7.69 mg/g, demonstrating good insect growth inhibition properties. The highest GI_{50} of 13.60 mg/g was shown by the combination of *Isodon* and *Goniothalamus* in the ratio of 70: 30.

Both *Isodon* and *Goniothalamus*, singly without combination, gave nearly similar grain protection against rice weevils, as evident from Table 1. A concentration-dependent association is evident in the produced regression graphs for all treatments, as shown in Figure 3. Similar dose-dependent bioactivity against different storage pests by using various crude plant powders was reported by various other researchers also[15-17].

Farmers around the world traditionally use native flora to protect harvested food grains from arthropod pests. These plants are many, depending upon the region in which they are available, and farmers have relied upon them to protect their harvested produce. Neem is one well-known plant that farmers of this country have used for centuries to guard their crops against storage pests in the field and granaries. Over the years, different plant powders have been examined and evaluated by various researchers in different grains for protection against many storage pests. The use of various flora and their parts –roots,

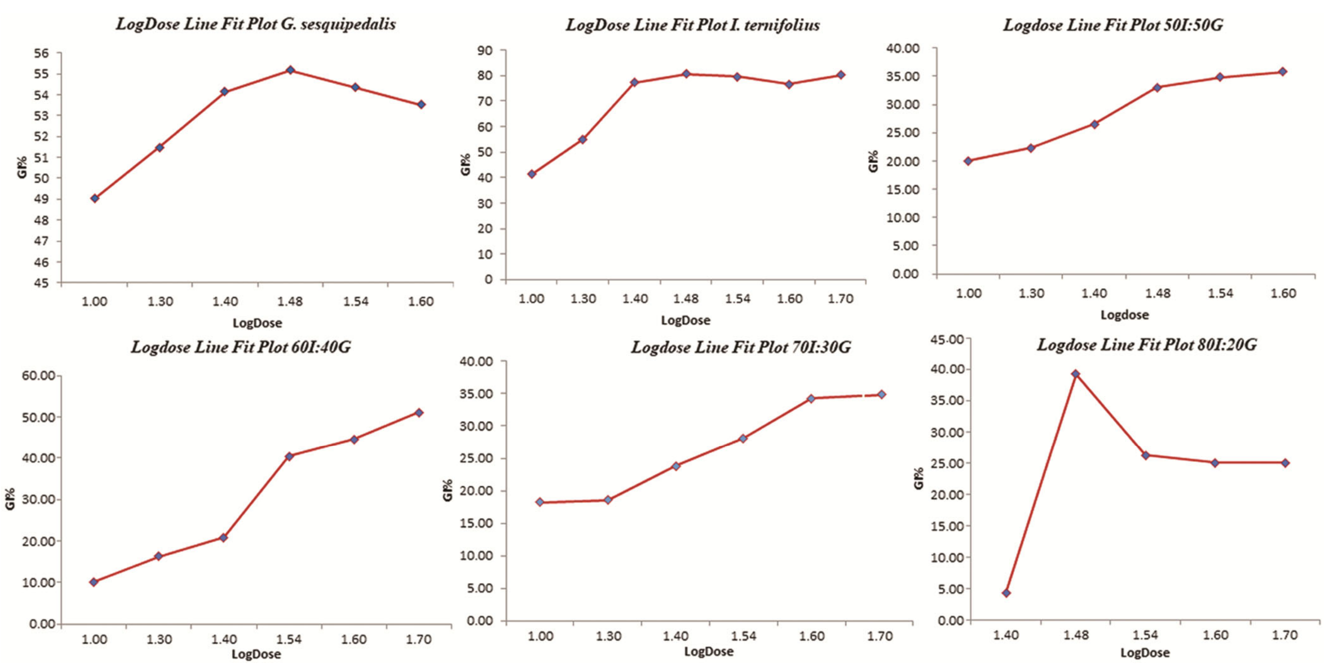

Fig. 3 — Log dose line fit plots for all treatments evaluated

Table 1 — Comparative growth inhibitory activity (GI_{50}) of various indigenous plant powders and their combinations in known fixed ratios

Plant Powder/ Combination	b±SE	Regression Equation	p-value	GI_{50} (mg/gm)	OE
I. ternifolius (local name: *khoiju*)	60.92±12.79	y=-17.14+60.92(x)	0.005	3.00	2
G. sesquipedalis (local name: leikhaam)	9.32±2.45	y=40.04+9.32(x)	0.01	2.92	1
50 Isodon:50 Goniothalamus (50I: 50G)	17.99±12.69	y=3.79+17.99(x)	0.008	9.97	5
60 Isodon:40 Goniothalamus (60I: 40G)	64.93±14.29	y=-59.95+64.93(x)	0.005	5.64	3
70 Isodon:30 Goniothalamus (70I: 30G)	19.72±2.53	y=-1.54+19.72(x)	0.0005	13.60	6
80 Isodon:20 Goniothalamus (80I: 20G)	56.70±23.65	y=-65.79+56.70(x)	0.06	7.69	4

OE: Order of Efficacy

leaves, seeds, kernels, bark, essential oils and extracts from plant parts have been widely studied and well-documented to reduce oviposition by insects, seed/grain damage and even inhibit adult emergence in different storage pests. Plant derivatives and their solvent extracts were also determined to reduce survival rates of larvae and pupae and inhibit normal adult emergence[18,19].

Schmutterer (1990) documented that the application of neem seed kernel powder at a concentration of 1.0-2.0% resulted in a significant reduction in storage pest incidence in stored cereal grains for a substantial duration[20].

Plant powders from *Chromolaena odorata, Calotropis procera*, *Datura alba* and neem were evaluated against *R. dominica* on rice grains at 2.5 and 5.0%, and all the treatments were reported to be effective in reducing adult emergence from the treated grains[21].

The efficacy of powdered *Momordica charantia* leaves against *Callosobruchus maculatus* was evaluated for contact toxicity and oviposition deterrence. After 96 h of treatment, all the doses of plant powders examined (0.2, 0.4, 0.6, and 0.8 g) per 20 g of stored cowpea seeds showed a mortality rate above 75%. The highest insect mortality rate was 85% when using a dosage of 0.8 g. This dosage did not show a significant difference ($p<0.05$) in its effect compared to dosages of 0.2 g, 0.4 g, and 0.6 g. The oviposition by the insect was also observed to be reduced significantly[22].

More recently, in 2022, crude plant powders of *Chromolaena odorata*, *Azadirachta indica* and *Calotropis procera* were evaluated for biocidal activity against *C. maculatus*, and all the plant powders tested demonstrated significant insecticidal potency on the insect pest[17].

Not only plant powders but plant parts extracted in various organic solvents were also evaluated and found efficacy in reducing pest damage and even in progeny reduction against *S. oryzae*. Ethanol extracts from six plants, namely *Melia azedarach*, *Myrtus communis*, *Mentha longifolia*, *Pegnum harmala*, and *Cymbopogon citratus*, were evaluated for their insecticidal capabilities against the rice weevil, *S. oryzae*. The findings indicated that all examined plant species exhibited repellant and mortal effects against the insect to varied extents in comparison with the untreated control[23].

In another study, extract of *Piper nigrum* significantly repelled *S. oryzae* (92.0%) compared to *Jatropha curcas* extracts (69.6%). The mortality rate of *P. nigrum* was highest in petroleum ether (LC_{50}=1.61 μL/g) followed by chloroform extracts (LC_{50}=1.70 μL/g) than those of petroleum ether extracts (LC_{50}=6.82 μL/g) of *J. curcas* (99.56, 93.56, and 66.00%, respectively). Petroleum ether and chloroform extracts of *P. nigrum*, as well as petroleum ether extract of *J. curcas*, exhibited potent antifeedant properties and significantly reduced progeny production. All treatments resulted in the suppression of F1 adults at the lowest concentration (2 μL/g), with zero production of F1. Our current study also demonstrated a reduction in progeny production by rice weevil adults[24].

Recently in 2018, the plant powders and ethanolic extracts of *Cleistopholis patens* and *Eugenia aromatica* were evaluated against maize weevil, *S. zeamais* in stored maize. The highest mortality was obtained with a 2.5 g concentration of *C. patens* powder at 96 h after treatment followed by *E. aromatica* powder. Seed damage brought by weevil feeding and resulting percentage damage on stored maize grains were reduced by using plant powders of both plants[15].

The current study also parallels the discoveries and conclusions of Devi *et al.* (2014). The efficacy of plant powders derived from *Phlogocanthus thyrisiflorus, Parthenium hysterophorus*, *Melia azedarach, A. indica, Zanthoxylum acanthopodium* and *Vitex trifolia*, was examined in terms of their impact on mortality, rate of adult emergence, and grain damage caused by the rice weevil, *S. oryzae*, on rice grains. The highest average mortality was observed in *M. azedarach* (80.54%) > *Z. acanthopodium* (70.74%) > *A. indica* (70.74%) at 35 days after treatment. According to Devi *et al.* (2014), these three plants have the potential to safeguard stored rice from *S. oryzae* infestations[25]. The powdered extract of *Alstonia, Hyptis suaveolens* and *Tephrosia vogelii*, protected wheat grains under storage from Lesser grain borer, *Rhyzopertha dominica*. The three plant powders significantly reduced the number of laid eggs, the percentage of emerging insects and increased insect mortality in the treated grains with control. Our study also finds resonance with the findings of Negbenebor and Nura, (2020)[16].

The efficacy of turmeric, garlic, and curry leaf powders and oils in controlling the stored maize weevil, *S. zeamais*, was assessed. The results showed that the oils and powders derived from curry leaves and turmeric were highly effective, causing a significant mortality rate of 82.50% and 81%, respectively. Garlic powder was also found to be equally effective, resulting in a mortality rate of 72.00% for the weevils. There was a significant decrease in the number of exit holes and the percentage of seed damage in maize seeds treated with concentrations of 0.50% and 1.00%. All the evaluated plant powders showed a decrease in weight loss in the stored maize seeds as compared to the control[26].

Effect on seed germination

The germination percentage of rice seeds at different doses after treatment is given in Table 2. The average germination percentage of rice seeds treated with *G. sesquipedalis* plant powder for three months was 82% and that with *I. ternifolius* was 86%. Untreated rice seeds were found to record 80% germination. The viability of seeds treated with *G. sesquipedalis* and *I. ternifolius* were found to be both enhanced. The seeds treated with a combination of both *G. sesquipedalis* and *I. ternifolius* failed to germinate completely. Seeds that are heavily infested by insects show less germination and may become unsuitable for sowing. With the increasing developmental stage of *S. zeamais* infesting maize grains, there was a corresponding decrease in germination[27]. Neem-treated wheat samples exhibited higher seed germination rates (87.50%, 85.00%, and 81.00%) compared to the untreated control[28]. Seed treatment with Neem leaf and kernel powders, Eucalyptus, Sarifa and Lantana leaf powders did not decrease seed germination for stored paddy and wheat seeds[29]. For sowing purposes, a treatment for protection against storage pests should not hinder the germination of seeds. However, complete inhibition of germination of rice seeds was observed in the plant powder combinations of *Goniothalmus* and *Isodon*, while providing good protection against the storage pest. Hence, farmers can use this plant powder mixture to safeguard against pests; however, they should not use the treatment combination of these two plants to treat seeds kept for sowing purpose in the next season.

Table 2 — Germination percentage after treatment with different doses of plant powders

	Goniothalamus		*Isodon*	
Doses per 50 g seeds	1st count	Final count	1st count	Final count
	60%	83%	60%	83%
0.5 g	63%	86%	63%	86%
1 g	76%	93%	76%	93%
1.25 g	70%	86%	70%	86%
1.5 g	53%	86%	53%	86%
1.75 g	66%	83%	66%	83%
2.0 g	56%	83%	56%	83%
2.5 g	63%	83%	60%	83%
average	-	82%	-	86%
CONTROL	80%	80%	80%	80%
Goniothalamus + *Isodon*	Complete inhibition of germination			

Mode of action

The crude extracts and compounds of certain plant families such as Asteraceae, Annonaceae, Lamiaceae, Meliaceae, Piperaceae, Rutaceae have shown wide variety of effects against various insects such as toxicity[30], antifeedant activity[31-33], presence of insect growth regulators[34,35], oviposition deterrence[36,37], suppressing mating call[38] and restricting fecundity and fertility[35].

The plants used in the study are considered among the most promising plant families with grain protection properties[39], in addition to other anti-insect abilities. *Isodon* is one of the important genera under Lamiaceae, and *Goniothalamus* is a prehistoric taxon of flowering plants under the genus Annonacea consisting of about 160 species, of which only six species of *Goniothalamus* have been applied as ethnomedicines in Asia[40].

I. ternifolius is rich in diterpenoids[41], lignans and phenylethanoid[42]. While phytochemical studies on *Goniothalamus* species revealed various constituent compounds, especially styrylpyrone derivatives, quinoline and isoquinoline alkaloids, phenanthrene lactones, terpenes, acetogenins and flavonoids[40]. Bioactive secondary plant metabolites are crucial in plant defence systems against insect herbivory. The plants examined in our study are an abundant source of diverse bioactive chemicals that are known to be effective against insects. These compounds may have a substantial impact on lowering the population growth of rice weevils by reducing the rate of oviposition through reduced fertility and fecundity in our current investigation[35]. The combination of various plant bioactive metabolites likely resulted in a powerful toxic blend that significantly enhanced its effectiveness against insects through synergistic effects. Crude plant extracts, including plant powders are mostly complex mixtures of bioactive compounds. Our results agree with those of Berenbaum and Neal, 1985, Berenbaum *et al*., 1991; Chen *et al*., 1995 who advocated the positive benefits of using crude mixtures comprising of many active compounds for pest control as natural mixtures may act synergistically, and may show greater overall bioactivity compared to the individual constituents[43-45]. Practically too, the crude extracts are relatively easier to prepare and simpler when the raw plant materials are available. The development of insect resistance is also less likely to develop with such complex crude compositions as the responses of insect chemical receptors to a specific stimulus may be modified by the presence of various plant constituents.

Conclusion

Both the plant species have abundant and diverse bioactive principles that might be imparting growth-inhibiting properties in insects. *Goniothalamus* and *Isodon* alone or in various combinations can be safely used for storage pest management that is sustainable and eco-friendly. Treatment with these plant powders singly without combination enhances seed germination, too. Farmers can easily use such technologies from plants that are readily available and safe for health to store harvested foodgrains in their own homes. Using these plants for storage pest management in traditional granaries in Manipur is a widespread practice and is almost done in every household by almost every tribe of Manipur. It is not an ITK that was practiced in earlier times and has lost its relevance. The Indigenous Traditional Knowledge (ITKs) are valuable and beneficial assets for farmers with limited resources. It is essential to conduct thorough scientific research and improvement of the ITKs while also documenting them appropriately. This is necessary to effectively implement them in the context of organic farming. Scientific validation of traditional knowledge is a crucial process for establishing the legitimacy of indigenous culture in the global community, empowering the indigenous people and making a meaningful contribution to contemporary research and environmental conservation.

Significance

This study validates the ancient practice of protecting stored foodgrains from storage pests in the highly humid climate of Manipur using *G. sesquipedalis* and *I. ternifolius*. Few scientific documents validate the grain protective ability against insect pests of certain Indigenous plants used traditionally by farmers, and it is significant as it highlights the path ahead for scientific attention and focused research combining ancient folk know-how and experience with a scientific temperament to create a fusion for a sustainable, improved, environment-friendly yet modern technology. Plants and its derivatives offer a safer alternative to chemical pesticides while being flexible enough to be used alone in organic cultivation or to be used in rotation with chemical pesticides or for easy integration into existing IPM strategies for crop pest management. These solutions are environmentally benign, sustainable, and easily accessible at a low cost for small, medium, and subsistence farmers in developing nations. They help to safeguard and maintain the results of their hard work. Given the increasing resistance of storage insects to chemical pesticides and the rising demand from health-conscious and environmentally aware consumers for pesticide-free and sustainably sourced food products, traditional practices offer a sustainable approach to finding new and effective molecules that can be used as a foundation for synthetic pesticide chemistry.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

AN: Conceptualization, Methodology, Formal Analysis and Writing - original draft. RA, AB, IMS, SKS: Methodology, Resources, Review, Editing. LG & THSKS: Resources, Observation, Data Collection and Compilation, YPD: Technology Validation.

Data Availability

The data is submitted to Krishi Technology Portal of ICAR with approved Technology Code: 201637822474893

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