*Biological Forum – An International Journal* (SI-AAEBSSD-2021) 13(3b): 295-299(2021)

**ISSN No. (Print): 0975-1130**
**ISSN No. (Online): 2249-3239**

# Bioefficacy of *Zanthoxylum acanthopodium* and its Combination with *Plectranthus ternifolius* as a Grain Protectant against Rice Weevil, *Sitophilus oryzae*

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*(Received 01 July 2021, Accepted 25 September, 2021)*
*(Published by Research Trend, Website: www.researchtrend.net)*

**ABSTRACT: The bioefficacy of *Z. acanthopodium* was studied as a grain protectant against rice weevil, *Sitophilus oryzae*, alone and in combination with *Plectranthus ternifolius*. Rice weevil was found to be the most common and dominating insect pest of stored pulses under NE conditions. The population growth-inhibiting ability of the plant powders by way of suppressing the progeny production was evaluated. Fixed combination ratios of 50:50, 60:40, 70:30 and 80:20 of *Zanthoxylum* and *Plectranthus* were studied and evaluated. The $GI_{50}$ of all the plant powders were calculated, and *Z. acanthopodium* without any combination was found to have the lowest $GI_{50}$ of 3.03mg/gm. Among the combinations, *Zanthoxylum* and *Plectranthus* in 50:50 ratio gave the best population growth suppressing ability with $GI_{50}$ of 5.47mg/gm, and 80:20 ratio combination was found the least effective with $GI_{50}$ of 6.88 mg/gm. Singly or in combination, all the plant powders gave good protection against rice we evil with very low $GI_{50}$. The seeds treated with only *Zanthoxylum* showed a higher germination rate of 85% compared to 80% in control, while combined powders inhibited seed germination.**

**Keywords:** rice weevil, grain protectant, progeny reduction, *Zanthoxylum, Plectranthus*, ITK, NE India

## INTRODUCTION

The latest United Nations 2019 Revision of World Population Prospects states that the global population reached an estimated 7.7 billion people worldwide in 2019, the medium-variant projection indicates that the global population could reach around 8.5 billion in 2030, 9.7 billion in 2050, and 10.9 billion in 2100 (UN, 2019). According to the United Nations World Food Programme (UNWFP), acute food insecurity affected 135 million people in 55 countries in 2019 and one in three – suffered from some form of malnutrition. The Global Nutrition Report (2021) reports that globally, 149.2 million children under five years of age are stunted, 45.4 million are wasted, and 38.9 million are overweight. Out of 2.2 billion people, 40% of all men and women are now overweight or obese. It further states that an estimated 155 million people are being pushed into extreme poverty globally due to the pandemic. People who are obese or have other diet-related chronic diseases are more vulnerable to Covid-19, which certainly adds to the challenge of meeting global nutrition targets. Providing adequate food and dietary proteins to an increasing global population without harming the environment is a challenge.

Even as humanity face a food security and nutrition crisis aggravated by a pandemic, food production has always been under threat of pests and diseases since the domestication of crop plants. Agricultural food production and stored products are directly impacted by insect infestation, which may account for 20-30% production loss and complete loss, according to De Geyter *et.al.*, (2007). Therefore, there is a great necessity to reduce post-harvest food losses to ensure global food security sustainably. Therefore, post-harvest protection of grains against insect pests is of paramount importance to ensure food security.

Across a few decades' experts believe that the focus of agricultural policy worldwide has focused on increasing agricultural production and productivity while managing field pests and pathogens; however, post-harvest losses have hardly been given due emphasis. Less than 5% of research funding is accorded to it (Bourne, 1977; Greeley, 1986, Kitinoja *et al.*, 2011 and Pantenius, 1988). Gustavsson *et al.*, (2011)report that on a global scale, about one-third of the total food produced, about 1.3-billion-tonnes worth about $1 trillion, is lost every year due to post-harvest losses.

While moisture content and temperature are the most crucial abiotic factors affecting the storage life, insect pests are considered the most important among all the biotic factors and cause considerable losses in the grains (30%–40%) (Tapondjou *et al.*, 2002; Boxall, 2001; Abbas *et al.,* 2014). With the phasing out Methyl bromide used for chemical fumigation of stored grains and sole reliance on phosphine gas, the resistance of stored-grain insects to phosphine has now been discovered in more than 45countries (Bell and Wilson, 1995; Chaudhry, 1995). An estimated 70% of total food grains produced in the country is handled at farmers' level while the rest is stored with Food Corporation of India, Central and State Warehousing Corporations. Our survey among North East India (NE India) farmers found that they do not use any chemical treatment/fumigation in their traditional granaries. Instead, they used locally available indigenous plants to protect their grains and stored seeds from storage insect pests. A vast wealth of traditional knowledge exists among the people which are still popular and widely believed. These plant parts are either mixed along with grains, seeds or these plant parts were hanged inside the granaries or burned to generate smoke before storing a new harvest (Prakash *et al.*, 2016). The search for plant-derived compounds could be a valuable source in developing bio-pesticides for sustainable and healthy agriculture (Silva *et al.*, 2012).

The most common pest in stored rice grains is the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae). Interestingly, it was also found to be the dominant storage insect pest of stored pulses under NE Indian conditions due to high humidity. It is a cosmopolitan pest causing considerable qualitative and quantitative loss during storage. The present study focuses on an indigenous plant, *Zanthoxylum acanthopodium* (local name: *mukthroobi thingkhang-panbi*), which was traditionally used as a grain protectant against storage pestsin Manipur. The grain protective ability of *Z. Acanthopodium* by suppressing the insect population's growth is studied individually and in combinations of fixed ratios with another indigenous plant, *Plectranthus ternifolius* (local name: *khoiju*). Although these plants were used traditionally by the people, sometimes alone or in combination, no proper scientific study has been done to validate their use. Despite being recognized as a treasure house of ITK (Indigenous Traditional Knowledge), scientific documentation and validation of folk wisdom in the NE Region need more study and research. Ansari *et al.*, 2021 observes that indigenous traditional knowledge is getting lost with time and that documenting them is crucial for creating a foundation of modern technology. Scientific validation of ITK is the next important step to blending folk wisdom and scientific approach for creating a new effective technology that is also sustainable and eco-friendly. This study attempts to highlight this folk practice used by ethnic people to protect their foodgrains from insect damage.

## MATERIALS AND METHODS

The work was conducted at ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal-795004, Manipur, from 2018 to 2020.

The leaves of *Z. acanthopodium* were identified and collected. It was then shade dried and ground into fine powders. An experiment was laid out to study the bioefficacy of these indigenous plants in deterring the population growth of rice weevil, *S. oryzae*. A pair of rice weevils, i.e., male and female of uniform size and growth, were released in treated rice grains. The insects were obtained from the insect culture already available and maintained with the Entomology section of ICAR, Manipur Centre. Different doses of the plant powders (shade dried and finely ground) were mixed with rice grains, and observations were recorded every week for growth in the insect population. Altogether seven treatment doses were tested i.e. 0.5, 1.0, 1.25, 1.50, 1.75, 2.0 and 2.5 gm of crude plant powder. Different doses of finely ground shade dried leaves were mixed with 50 gm of rice grain samples in three replicates, and observation was recorded every week. Observation on population growth was recorded by counting the progeny from the paired insects. As farmers keep a part of their harvested produce for use as seeds for the next season, observations were kept for 12 weeks.

Similarly, the leaves of *P. ternifolius* was also shade-dried and ground into fine powder. It was then mixed with ground powder of *Z. Acanthopodium* in fixed ratios of 50:50, 60:40, 70:30 and 80:20. The whole procedure of releasing paired weevils into treated grains and recording weekly observations for three months was repeated for the plant powder combination of *Zanthoxylum* and *Plectranthus*. Untreated rice grains were kept as a control in both individual and combination treatments.

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

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

Regression analysis was done between log dose and the percentage growth inhibited in the treated grains using Microsoft Excel. For each treatment, regression equations were generated from which the $GI_{50,}$ i.e., the dose in which the growth of half the population of insects was inhibited, were calculated. The study on population growth-inhibiting ability was evaluated on plain white rice grains of rice variety RCM-12. In addition, the effect of these plants on seed health was also studied on rice seeds of the same variety. Rice seeds were treated with finely ground powders at the same doses used for treating the grains and kept for a period of three months. Germination tests were then performed on the treated rice seeds through the petri-dish method and germination paper method. For each technique, ten seeds were selected randomly in triplicates, and a germination test was conducted. The first count of germinated seeds was taken on the $5^{th}$ day, and the following final count was taken seven days later. The entire work was conducted at ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal-795004, Manipur, from 2018 to 2020.

## RESULTS AND DISCUSSION

The experiment was designed keeping in view how farmers use plant parts/products/powders to protect against storage pests in their homes/granaries and how they would prefer to adopt and use eco-friendly technology in an easy, convenient manner. The moisture content of the RCM-12 rice grains used for the study was measured using a Grain Analyzer before initiating the experiment and had an average moisture content of 12.65%. This is in the recommended range of 12-13% for most cereals, as Lipinski *et al.*, (2013) suggested. The observations involving the effect of the evaluated plants as grain protectants against rice weevil is shown in Table 1 and Table 2. Table 1 shows the mean insect population recorded for each treatment, i.e., plant evaluated in single and combined ratios. Table 2 shows the comparative efficacy of each treatment through their respective $GI_{50}$.

All the treatments showed remarkable insect population growth suppressing ability. The $GI_{50}$ ranged from 3.03 mg/gm to 6.88 mg/gm. The lowest $GI_{50}$ was exhibited by the rice grains treated only with *Z. acanthopodium* and was found to be the best among all the treatments in suppressing insect population growth. All the remaining treatment combinations of *Zanthoxylum* and *Plectranthus* also gave good results in reducing the insect population growth. The highest $GI_{50}$ of 6.88 mg/gm was shown by *Zanthoxylum* and *Plectranthus* combined in the ratio of 80: 20. Although it was the least effective of all the treatments, both singly and in combination, it may be considered an effective dose due to its low GI50.It is evident from Table 2 that *Zanthoxylum* alone was a better grain protectant than in combination with *Plectranthus*. In all the treatments, a dose-dependent relationship can be seen in the regression graphs plotted, as shown in Fig. 1.

**Table 1: Average population growth of insect population observed in each treatment for every dose evaluated.**

| **g per 50g seeds** | ***Zanthoxylum*** | ***Zanthoxylum: Plectranthus*** | ***Zanthoxylum: Plectranthus*** | ***Zanthoxylum: Plectranthus*** | ***Zanthoxylum: Plectranthus*** |
|---|---|---|---|---|---|
| | | **50:50** | **60:40** | **70:30** | **80:20** |
| **0.5** | 8.29 | 10.36 | 9.33 | 9.25 | 11.75 |
| **1** | 8.10 | 7.14 | 7.89 | 7.69 | 9.67 |
| **1.25** | 7.52 | 6.97 | 7.50 | 6.97 | 9.83 |
| **1.5** | 7.43 | 6.61 | 7.19 | 6.75 | 9.58 |
| **1.75** | 2.00 | 6.36 | 6.92 | 6.58 | 8.61 |
| **2** | 7.38 | 6.33 | 6.22 | 6.31 | 8.06 |
| **2.5** | 7.29 | 5.78 | 6.06 | 6.28 | 5.83 |
| **control** | 23.27 | 10.77 | 10.77 | 10.77 | 10.77 |

**Table 2: Comparative growth inhibitory activity ($GI_{50}$) of various indigenous plant powders and their combinations in known fixed ratios.**

| Plant Powder/ Combination | df | b±SE | Regression Equation | $GI_{50}$ (mg/gm) | OE |
|---|---|---|---|---|---|
| ***Zanthoxylum*** | 4 | 6.76 ± 2.45 | y=57.50+6.76(x) | 3.03 | 1 |
| ***Zanthoxylum: Plectranthus 50:50*** | 5 | 58.09±4.70 | y=-48.89+58.09x | 5.47 | 2 |
| ***Zanthoxylum:Plectranthus60:40*** | 5 | 44.33±1.41 | y=-31.26+44.33x | 6.23 | 4 |
| ***Zanthoxylum:Plectranthus70:30*** | 5 | 41.60±2.20 | y=-25.65+41.60x | 6.17 | 3 |
| ***Zanthoxylum: Plectranthus 80:20*** | 5 | 67.62±7.40 | y=-80.80+67.62x | 6.88 | 5 |
| OE: Order of Efficacy | | | | | |

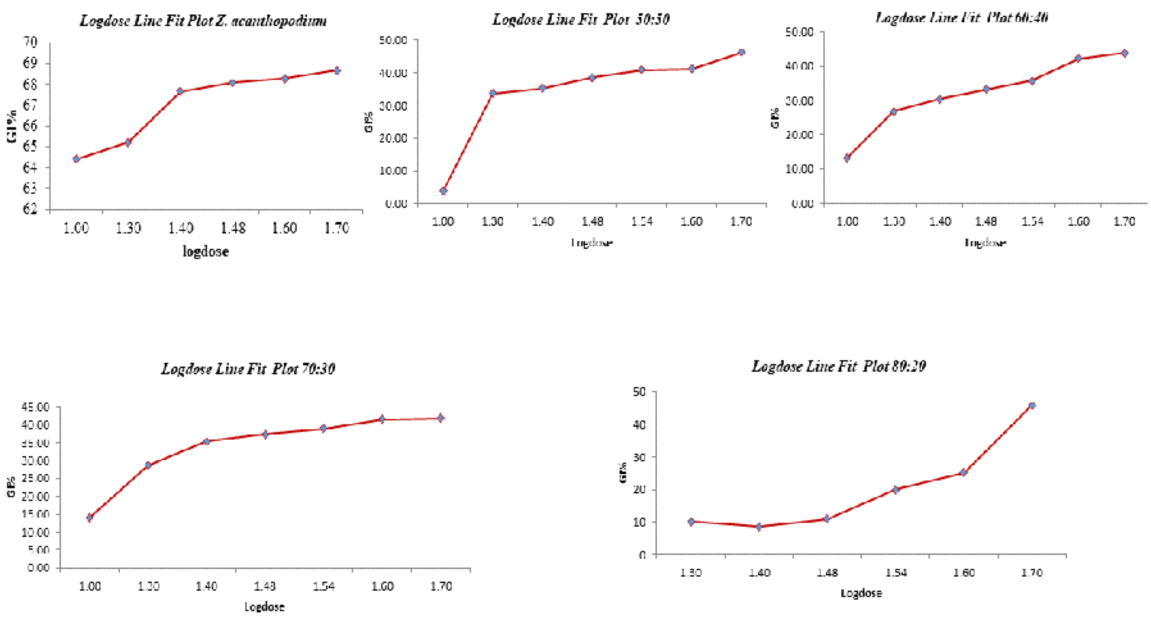


**Fig 1: Log Dose Line Fit Plots for all the treatments evaluated**

Given that in India, more than 70% of total food grains produced are handled and stored by farmers, with only 30% held with govt. Godowns, traditionally Indian farmers have relied on many plants to protect their food grains from insect pests after harvest, especially neem. Researchers around the globe have well-documented the use of various plants and their parts, viz. leaf, bark, seed powder, or oil extracts, when mixed with the stored grains. As a result, they reduced the rates of seed damage, egg-laying by insects and inhibiting adult emergence in different storage grain pests (Shaaya *et al.*, 1997; Keita *et al.*, 2001; Tapondjou *et al.*, 2002 and Jotwani and Sircar, 1965). Koul *et al*., (2008) also observed that plant derivatives reduced larvae and pupae' survival rates and inhibited adult emergence.

In India, Jotwani and Sircar (1965) first tested and reported that neem kernel powder mixed at 1.0 or 2.0 per cent with wheat grains protected the treated grains against *Rhyzopertha dominica* and *S. Oryzae* for more than 300 days. Dried leaves of *neem* gave adequate protection against insects when mixed with stored grains was validated by Yadava and Bhatnagar in 1987. Neem seed kernel powder @ 0.5 per cent was the most effective in providing complete protection against *Callosobruchus maculatus* in green gram by Singh *et al.*, 1996. Schmutterer (1990) also reported *neem* seed kernel powder at 1.0-2.0 per cent decreased pest infestation in stored cereal grains for a considerable period. Similarly, Mohan *et al.*, (1990) reported no grain damage by *S. oryzae* after treatment of maize with deoiled *neem* seed kernel powder at 0.1 per cent. Rajashekar *et al.*, (2010) observed that root powder extracts of *Decalepis hamiltonii*, when mixed with stored grains, gave protection against variousstored grain insect pests. Singh (2016) found neem powder and oil formulations as the most effective treatment against *R. dominica* and *S.oryzae* on stored wheat among different powder formulations of neem, castor, dharek, and oil formulations of neem, eucalyptus, and castor evaluated. According to Talukder (2006), botanical pesticides showed detrimental effects on the growth and development of insects, weight reduction in larva, pupa and adult stages and lengthening their development stages. Jacob and Sheila (1993) also evaluated plant powders from *Datura alba*, *Calotropis procera*, *Chromolaena odorata* and neem at 2.5and 5.0 per cent against *R. dominica* on rice grains. They reported all the treatments were effective in significantly reducing the number of adults emerging from the grains.

Yadu *et.al.*, (2000) evaluated neem kernel powder, neem leaf powder, eucalyptus leaf powder, sarifa leaf powder and lantana leaf powder at 1.0 and 2.0 per cent (w/w) for recording their adverse effects on the development of *S. cerealella* in stored maize and paddy.Neemkernel powder was found to be the most effective as it registered less grain damage and adult emergence whereaslantana leaf powder was the least effective. Mishra and Pandey (2014), reported neem leaf powder at 1.0 per cent (w/w) as themost effective against *S. oryzae* based on low grain damage(5.36, 8.43 and 16.02%) and weight loss (5.36, 7.87 and13.13%) over the untreated control with high grain damage(9.20, 18.55 and 29.60%) and weight loss (8.72, 14.40 and20.99%) in stored wheat, respectively, at 30, 60 and 90 DAT(days after treatment).In our findings, treatment with *Z. acanthopodium* with rice grains, singly and together with *Plectranthus* could protect grains from severe infestation by *S. Oryzae* for 90DAT. Devi *et.al*., (2014) evaluated plant powders prepared from *Melia azedarach, Parthenium hysterophorus, Phlogocanthus thyrsiflorus, Vitex trifolia, Zanthoxylum acanthopodium* and *Azadirachta indica* in Manipur for their efficacy on mortality, rate of adult emergence, grain damage effect against rice weevil, *S. oryzae* on rice grain. Plant powder of *M. azedarach* recorded the highest mean mortality of 80.54% at 35 DAT followed by *Z. acanthopodium* and *A. indica.* Both showed 70.74% mortality, whereas *P. hysterophorus* and *P. thyrsiflorus* were found less effective with 56.11 % mortality, followed by *Vitex trifolia* with 36.66% mortality. The study found *A. indica* plant powder to be highly effective in prohibiting the adult emergence and reducing grain damage per centover other treatments. Devi *et.al*., (2014)concluded that *M. azedarach, A. indica* and *Z. acanthopodium* could be used for the protection of stored rice from infestations of *S. oryzae.* The present study also finds similarities with the findings and conclusions of Devi *et al.*, (2014).

It is well-known that stored seeds/ grains heavily infested by insects have reduced germination rates and are sometimes entirely rendered completely unsuitable for sowing. Santos *et.al.*, (1990) reported from Brazil that *S. Zeamais* infestation in maize grains led to a reduction in germination with the increasing developmental stage of the insects, from 13% at the egg stage to 93% at the adult stage for *S. zeamais.* Earlier Okiwelu *et al*., (1987) recorded a high level of moisture, combined with a decrease in germination ability of maize due to infestation by *S. zeamais* in Nigeria. Therefore, any treatment for protection against stored pests should render protection against insect damage and should not negatively affect the germination of the seeds. After treatment for three months with *Zanthoxylum* plant powders, the average germination percentage was 85% while it was 80% in control. The viability of rice seeds treated only with *Zanthoxylum* was not adversely affected but was found to be enhanced. Mishra and Pandey (2014) also recorded higher seed germination (87.50, 85.00 and 81.00%) in neem treated samples over the untreated control (92.00, 71.25 and 54.37%), respectively, at 30, 60 and 90 DAT. Yadu *et al.*, (2000) observed no impaired seed germination of stored paddy and wheat after seed treatment with neem kernel powder, neem leaf powder, eucalyptus leaf powder, sarifa leaf powder and lantana leaf powder.

However, the plant powder combinations of *Zanthoxylum* and *Plectranthus* completely inhibited germination of the rice seeds, although giving good protection against the pest. Hence, it is not advisable for farmers to treat seeds kept for sowing for the next season.

The plants used in this study are well-documented ethnomedicinal plants with widespread use and popularity among NE Indian folklore and traditional knowledge. They belong to the family of Rutaceae (*Zanthoxylum*) and Lamiaceae (*Plectranthus*) and come under the most promising plant families with grain protecting abilities, as observed by Jacobson (1989). In the face of growing resistance to chemical pesticides in storage insect pests and a growing consumer base preferring pesticide-free organic food, botanicals and plant products provide not only safer alternatives to chemical pesticides but also are readily available, eco-friendly, sustainable and economical solutions to subsistence, small and medium farmers in developing countries to protect and preserve the fruits of their hard labour. *Zanthoxylum* alone or combined with *Plectranthus* can be safely used as an eco-friendly storage pest management strategy.

## CONCLUSION

The study addresses multiple and vital issues of varying dimensions. Post-harvest loss of food grains is an important yet neglected area of agricultural policy and research, according to many experts, as stated earlier. It has received less attention than studies on crop losses due to pests and diseases in the field. This study also scientifically documents and validates a traditional knowledge practised by an indigenous population in NE India in the often-neglectedarea of post-harvest grain loss due to insect pests. It is common knowledge that due to time and growing modernization, the vast reserve of ITK stored and passed down from generation to generation is getting lost. It paves the way for future research and scientific attention to combine ancient folk knowledge with a scientific approach to blend and fuse them to create better, modern, sustainable, environmentally friendly technology. Plant or plant-derived products can be easily integrated into IPM strategies and conventional pesticides or alone in organic farming. For post-harvest storage of food grains at farmers' homes, such technologies are readily available and safe alternatives to combat stored grain pests.

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