

# Synergistic Pesticidal Potential of Linarin and Salannin–Based Botanical Formulation Against *Thrips tabaci* and *Sitophilus oryzae*

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## ABSTRACT

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## Introduction

Onion thrips, *Thrips tabaci* Lindeman, is a major pest of onion and other *Allium* crops, causing severe yield and quality losses by feeding on foliage and transmitting plant pathogens [Frewin et al., 2021]. Thrips infestations lead to silvering, curling, and drying of leaves, ultimately reducing bulb size and compromising market value [Frewin et al., 2021]. Conventional management of onion thrips and storage pests such as rice weevil, *Sitophilus oryzae*, still relies heavily on synthetic insecticides, leading to problems of resistance, residue accumulation, and environmental contamination [Isman, 2006]. Increasing regulatory restrictions and consumer demand for residue-free food have intensified the search for botanical biopesticides as safer alternatives [Isman, 2006].

Botanical insecticides derived from plants such as *Azadirachta indica* and *Chrysanthemum* species are known to possess insecticidal, repellent, and antifeedant properties and are increasingly explored in modern pest management [Schmutterer, 2013; Simmonds, 2003]. Neem-based limonoids including azadirachtin, salannin, and related triterpenoids act by disrupting insect growth, deterring feeding, and impairing development, making them suitable candidates for integrated pest management (IPM) [Biswas et al., 2002; Subapriya & Nagini, 2005]. Salannin, a key limonoid in neem, exhibits strong antifeedant and insect growth–regulating activity, prolonging larval stages and increasing mortality in

various insect species [Govindachari et al., 1996; Roy et al., 2010].

*Chrysanthemum* species are traditionally recognized for their insecticidal pyrethrins, and recent investigations highlight additional bioactive flavonoids such as linarin with insecticidal and deterrent potential [Simmonds, 2003; P. Kumar et al., 2023]. Linarin-rich *Chrysanthemum* flower extracts obtained through hydro-alcoholic cold maceration have demonstrated significant insecticidal activity, supporting their use as botanical pesticides [M. Shaikh et al., 2025]. Botanical insecticides are generally biodegradable, show reduced non-target toxicity, and can be integrated with other control tactics in IPM programs [Isman, 2006]. Several field and laboratory studies have reported efficacy of neem formulations, garlic extract, entomopathogenic fungi, and other botanicals against onion thrips, highlighting the potential of plant-based solutions for this pest [Frewin et al., 2021; P. Kumar et al., 2023]. However, there is limited information on synergistic combinations of specific flavonoids and limonoids, particularly linarin with salannin, for simultaneous management of agricultural and storage pests.

The present study aimed to develop and evaluate a novel botanical biopesticide, “CryAza”, formulated as a synergistic blend of linarin from *Chrysanthemum morifolium* flowers and salannin from *Azadirachta indica* leaves for the control of onion thrips and rice weevil under laboratory conditions.

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## Materials and Methods

### Plant materials

Dried flowers of *Chrysanthemum morifolium* were used as the source of the flavonoid linarin, and dried leaves of *Azadirachta indica* (neem) were used as the source of the limonoid salannin. Fresh plant materials were collected

locally, authenticated morphologically, and subjected to shade drying at room temperature to preserve thermolabile constituents and ensure compound stability. The dried flowers and leaves were powdered using a clean mechanical grinder and stored in airtight containers until extraction

**Image 1**

*Chrysanthemum morifolium*  
Dried Leaves and It's Powder



**Image 2**

*Azadirachta indica*  
Dried Leaves and it's Powder

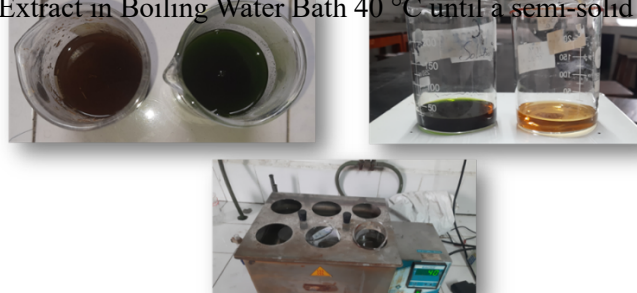


### 2.2 Extraction of bioactive compounds

Hydro-alcoholic cold maceration was employed to extract linarin and salannin from the powdered plant materials. For each plant, an appropriate quantity of dried powder was soaked in 70% ethanol, a widely used solvent that efficiently extracts both moderately polar flavonoids and limonoids. The mixtures were maintained at ambient temperature under occasional shaking for sufficient time to allow diffusion of bioactive constituents into the solvent. The macerates were filtered through muslin cloth followed by Whatman filter paper, and the combined filtrates were concentrated in a water bath at 40 °C until a semi-solid extract was obtained, thus minimizing thermal degradation. The concentrated extracts were transferred to amber-coloured vials and stored at 4 °C until further use to protect the compounds from light and oxidative degradation.

**Image 3**

Ethanollic Extract of *Chrysanthemum* Leaves and  
Concentration of Extract in Boiling Water Bath 40 °C until a semi-solid extract was obtained

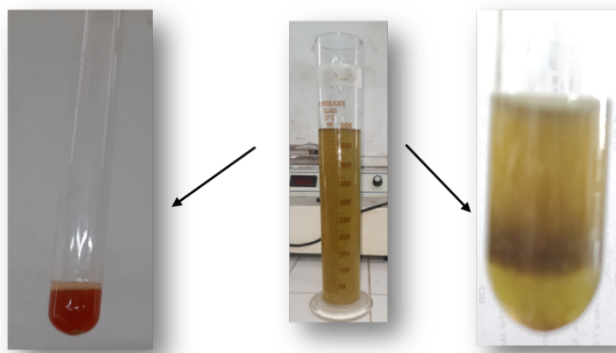


### 2.3 Phytochemical confirmation of linarin and salannin

Preliminary phytochemical confirmation of the target bioactive compounds was carried out using standard colour reactions. The presence of linarin, a flavonoid glycoside, was confirmed qualitatively by the Shinoda test, which produces a characteristic colour change in the presence of flavonoids. The presence of salannin and related triterpenoid limonoids was confirmed by the Salkowski test, which indicates steroidal and triterpenoid structures through a characteristic colour reaction with concentrated acids. Positive Shinoda and Salkowski tests for *Chrysanthemum* and neem extracts, respectively, confirmed successful extraction of the desired bioactive constituents for formulation.

**Image 4**

Confirmatory Tests for Presence of Bio-Active Compounds



Shinoda Positive Test  
for “Linarin”

Salkowski Positive  
Test for “Salannin”

#### 2.4 Formulation

The CryAza formulation was prepared by combining Chrysanthemum and neem powders in a defined ratio and extracting them together in 70% ethanol. Specifically, 2.5 g of Chrysanthemum dried flower powder and 5.0 g of neem dried leaf powder were separately dispersed in 50 mL of 70% ethanol each and then combined to obtain a total of 7.5 g plant material in 100 mL solvent. The combined mixture was subjected to maceration with intermittent shaking, followed by filtration to remove plant residues. The filtrate was concentrated in a boiling water bath at 40 °C for approximately 2.5 hours to obtain a semi-solid crude extract containing the synergistic blend of linarin and salannin. The final CryAza extract was stored in an amber-coloured reagent bottle at 4 °C until bioassay, and a stock solution was prepared at 250 mg/mL using appropriate solvent.

**Image 5**

Self made bioinsecticide spray “CryAza”



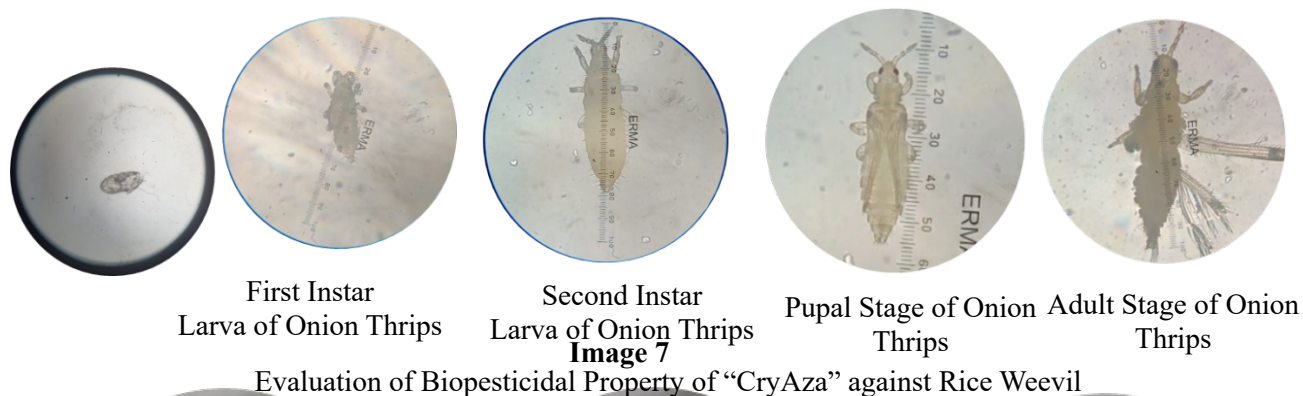
#### 2.5 Test insects

The target pest species included onion thrips (*Thrips tabaci*) as an agricultural pest and rice weevil (*Sitophilus oryzae*) as a storage pest. Onion thrips were collected from infested onion fields and maintained temporarily in clean Petri dishes lined with filter paper under laboratory conditions prior to bioassay. Rice weevils were obtained from infested stored grain and maintained in glass jars with whole grains to keep them active and healthy until experimentation. Representative developmental stages of onion thrips (egg, first instar larva, second instar larva, pupa and adult) were observed and measured using an ocular micrometer to document the life cycle under laboratory conditions, although mortality assays focused primarily on active motile stages.

**Image 6**

Measurement of different stages in life cycle  
of Onion thrips with the help of Ocular Micrometer

Onion Thrips Egg



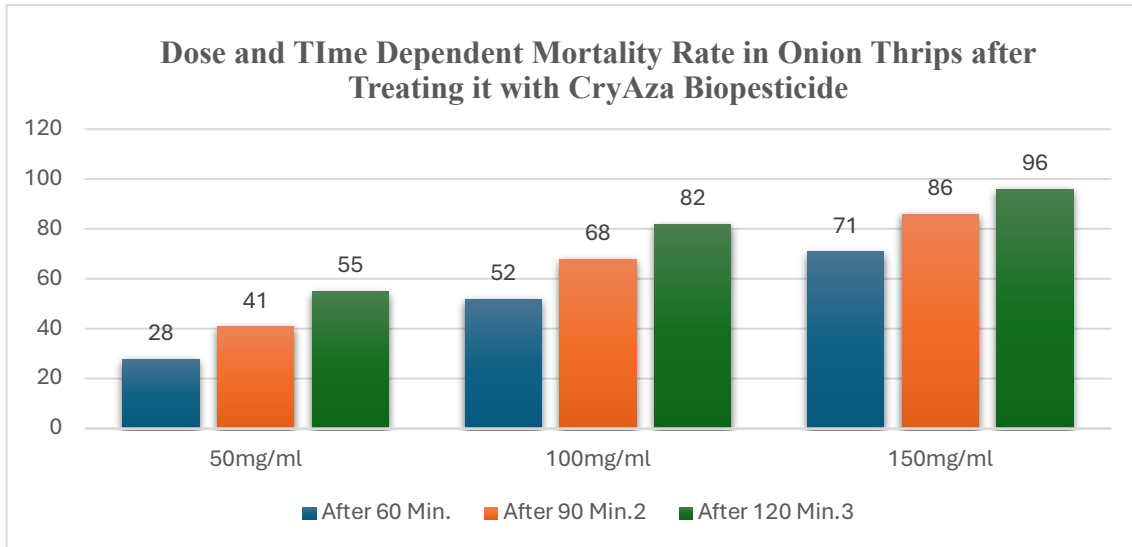
### 2.6 Bioassay design

Bioassays were conducted to evaluate the dose- and time-dependent insecticidal activity of CryAza against onion thrips and rice weevil. From the stock solution (250 mg/mL), three test concentrations were prepared: 50 mg/mL (low dose), 100 mg/mL (medium dose) and 150 mg/mL (high dose). For each insect species and each concentration, groups of insects were exposed to CryAza-treated surfaces or substrates, while maintaining untreated control groups where applicable. Each treatment was replicated in triplicate to ensure reliability of data and allow statistical analysis. Mortality was recorded at 60, 90 and 120 minutes after treatment, and insects showing no movement upon gentle prodding were recorded as dead.

**Table 1.** Dose dependent and time dependent mortality rate in Onion Thrips after treating with CryAza biopesticide

Sr.No.	Dose Conc.	After 60 Min.	After 90 Min.	After 120 Min.
1.	50mg/ml	28 ± 2.1	41 ± 2.4	55 ± 2.6
2.	100mg/ml	52 ± 2.5	68 ± 2.2	82 ± 2.1
3.	150mg/ml	71 ± 2.3	86 ± 2.0	96 ± 1.5

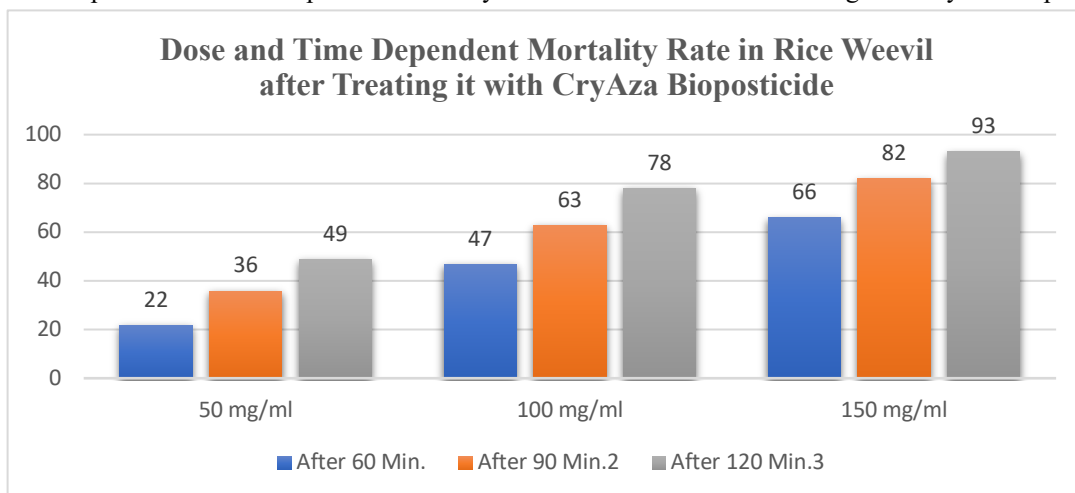
**Graph 1.** Dose dependent and time dependent mortality rate in Onion Thrips after treating with CryAza biopesticide



**Table 2.** Dose dependent and time dependent mortality rate in Rice Weevil after treating with CryAza biopesticide

Sr.No	Dose Conc.	After 60 Min.	After 90 Min.	After 120 Min.
1.	50mg/ml	22 ± 1.8	36 ± 2.0	49 ± 2.3
2.	100mg/ml	47 ± 2.2	63 ± 2.1	78 ± 2.0
3.	150mg/ml	66 ± 2.0	82 ± 1.9	93 ± 1.6

**Graph 2.** Dose dependent and time dependent mortality rate in Rice Weevil after treating with CryAza biopesticide



### 2.7 Statistical analysis

The percentage mortality data (mean ± standard deviation) for each concentration and exposure time were compiled separately for onion thrips and rice weevil. One-way analysis of variance (ANOVA) was performed for mortality at 120 minutes to determine whether differences among CryAza concentrations were statistically significant for each test insect. For onion thrips, the ANOVA table showed a between-treatments sum of squares of 3654.2 with 2 degrees of freedom, within-treatments sum of squares of 111.2 with 6 degrees of freedom, and an F-value of 98.6, indicating a highly significant effect of dose on mortality at 120 minutes ( $p < 0.05$ ). For rice weevil, the between-treatments sum of squares was 2897.6 with 2 degrees of freedom, within-treatments sum of squares was 117.1 with 6 degrees of freedom, and the resulting F-value of 74.2 similarly confirmed significant dose-dependent mortality ( $p < 0.05$ ).

Table 3. One Way ANOVA of Mortality of Onion Thrips after 120 Min.

Variation	SS	Df	MS	F-Value
Between Treatments	3654.2	2	1827.1	98.6
Within Treatments	111.2	6	18.5	
Total	3765.4	8		

Table 4. One Way ANOVA of Mortality of Rice Weevil after 120 Min.

Variation	SS	Df	MS	F-Value
Between Treatments	2897.6	2	1448.8	74.2
Within Treatments	117.1	6	19.5	
Total	3014.7	8		

One-way ANOVA revealed a statistically significant difference ( $p < 0.05$ ) in insect mortality among CryAza concentrations, confirming a dose-dependent insecticidal effect.

## Results and Discussion

### 3.1 Dose- and time-dependent mortality in onion thrips

CryAza induced clear dose- and time-dependent mortality in onion thrips under laboratory conditions. At the lowest concentration of 50 mg/mL, mean mortality increased from  $28 \pm 2.1\%$  at 60 minutes to  $41 \pm 2.4\%$  at 90 minutes and  $55 \pm 2.6\%$  at 120 minutes, demonstrating progressive toxicity with longer exposure. At 100 mg/mL, mortality values were substantially higher, reaching  $52 \pm 2.5\%$  at 60 minutes,  $68 \pm 2.2\%$  at 90 minutes and  $82 \pm 2.1\%$  at 120 minutes. The highest dose of 150 mg/mL produced the greatest mortality, with  $71 \pm 2.3\%$  at 60 minutes,  $86 \pm 2.0\%$  at 90 minutes and  $96 \pm 1.5\%$  at 120 minutes, approaching complete kill of the treated thrips population. The one-way ANOVA at 120 minutes confirmed that differences among concentrations were statistically significant ( $F = 98.6$ ,  $p < 0.05$ ), validating a strong dose-response relationship for CryAza against onion thrips.

These findings align with previous reports that botanical extracts, particularly neem-based products and other plant-derived insecticides, can effectively suppress thrips populations when applied at adequate concentrations and with sufficient contact time. The high mortality at 150 mg/mL suggests that the combination of linarin and salannin may exert both contact toxicity and feeding deterrence, resulting in rapid knockdown of thrips. Linarin-rich Chrysanthemum extracts have been shown to cause significant insecticidal effects, possibly by disrupting neuromuscular function or feeding behaviour. Salannin and related neem limonoids are known to interfere with insect feeding and development, and their

presence alongside linarin in CryAza likely enhances overall toxicity through synergistic mechanisms. The observed rapid mortality within a short period (120 minutes) indicates that CryAza has potential as a quick-acting spray formulation for thrips management in onion and other crops, particularly within organic or low-chemical-input systems.

### Dose- and time-dependent mortality in rice weevil

Similar to the results for onion thrips, CryAza produced marked dose- and time-dependent mortality in adult rice weevils. At 50 mg/mL, mean mortality increased from  $22 \pm 1.8\%$  at 60 minutes to  $36 \pm 2.0\%$  at 90 minutes and  $49 \pm 2.3\%$  at 120 minutes. At 100 mg/mL, mortality values rose to  $47 \pm 2.2\%$  at 60 minutes,  $63 \pm 2.1\%$  at 90 minutes and  $78 \pm 2.0\%$  at 120 minutes. The highest concentration of 150 mg/mL again produced the greatest effect, with  $66 \pm 2.0\%$  mortality at 60 minutes,  $82 \pm 1.9\%$  at 90 minutes and  $93 \pm 1.6\%$  at 120 minutes. The one-way ANOVA at 120 minutes showed an F-value of 74.2 ( $p < 0.05$ ), indicating a statistically significant difference among concentrations and confirming a strong dose-response pattern in rice weevils similar to that observed in thrips.

Botanical extracts, especially neem-based formulations, have previously demonstrated efficacy against stored-product pests including *Sitophilus oryzae*, acting through antifeedant effects, reduced fecundity and direct toxicity. The high mortality of rice weevils at the 150 mg/mL dose suggests that CryAza could be developed as a dual-purpose biopesticide for both field and storage pests, which is advantageous from a practical and economic perspective. The combination of a flavonoid (linarin) and

a limonoid (salannin) may broaden the spectrum of activity and reduce the likelihood of rapid resistance development, as multiple modes of action—such as feeding deterrence, growth disruption and nervous system interference—may operate simultaneously. The relatively quick mortality response within 2 hours also indicates potential for short-term curative application in stored grains, although longer-term residual efficacy and effects on grain quality should be investigated in future studies.

Synergistic potential and comparison with other botanicals

The formulation rationale for CryAza was to harness the synergistic potential of linarin from *Chrysanthemum morifolium* and salannin from *Azadirachta indica*, both of which have independently documented bioactivity against insects. Linarin-rich extracts display insecticidal properties, while neem limonoids, including salannin, function as potent antifeedants and insect growth regulators, especially in Hemiptera and Coleoptera. The high mortality rates observed at relatively moderate concentrations, and within a short exposure window, suggest that the combined formulation may be more effective than individual plant extracts used alone, a pattern consistent with synergistic interactions reported for other botanical mixtures.

Several recent studies have reported promising performance of botanicals and bio-rationals such as garlic extract, neem oil, azadirachtin, entomopathogenic fungi and spinosad-based products against thrips and other pests. However, many of these products are either relatively expensive, require complex formulations or are limited to specific pest groups or production systems. In contrast, CryAza is prepared using simple shade drying, cold maceration and low-temperature concentration steps, employing widely available plant materials and ethanol as a solvent, which makes it amenable to low-cost production at small or semi-industrial scale. The demonstrated activity against both onion thrips and rice weevil indicates that CryAza could serve as a versatile botanical biopesticide for integrated management of agricultural and storage pests, particularly in regions seeking eco-friendly alternatives to synthetic insecticides.

#### Limitations and future prospects

The present investigation was conducted under controlled laboratory conditions, which may not fully represent field or storage environments where factors such as temperature, humidity, UV exposure and complex pest communities can influence biopesticide performance. Further work is required to evaluate the field efficacy of CryAza against onion thrips in onion and

related crops, as well as its residual activity, phytotoxicity and compatibility with other IPM components. Similarly, extended storage trials are needed to assess its long-term protective effect against rice weevil and other storage pests, and to monitor any impacts on grain germination and organoleptic properties. Standardization of extraction, formulation stability studies, and detailed toxicological evaluation on non-target organisms, including natural enemies and mammals, will be necessary to progress towards commercialization and regulatory approval.

#### Conclusion

CryAza, a novel botanical biopesticide formulated from linarin-rich *Chrysanthemum morifolium* flowers and salannin-containing *Azadirachta indica* leaves, exhibited strong dose- and time-dependent insecticidal activity against onion thrips and rice weevil under laboratory conditions. Mortality increased consistently with rising CryAza concentrations and longer exposure periods, with maximum efficacy observed at 150 mg/mL after 120 minutes for both pests, and one-way ANOVA confirming statistically significant differences among doses. The simple, eco-friendly formulation process, combined with broad-spectrum activity against an important field pest and a key storage pest, indicates that CryAza holds promise as an effective, economical and sustainable biopesticide candidate for integration into pest management programs.

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#### Declaration

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