Biorational Management of Pulse Beetle (Callosobruchus chinensis L.) in Gram Seed

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors MAMH and MMU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SS, KA and TUW managed the analyses of the study. Author MH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The pulse beetle, Callosobruchus chinensis is one of the major pests in stored pulse causes 40-50% losses of pulses in storage. Experiments were conducted to study the efficacy of some selected biorational insecticides on percent mortality of beetle, percent weight loss of seeds, no. of eggs laid per female, percent hatchability, larval duration, pupal period and adult longevity of pulse beetle, Callosobruchus chinensis under laboratory condition. Among the different botanicals, neem oil (77%) was found the most effective showing 78% mortality of pulse beetle in direct method followed by Mahogany oil (69%) and karanja oil (62%). In case of indirect method, the highest percent mortality was recorded from karanja oil (37%) which was followed by Neem oil (33%) and Mahogany oil (33%). Among different microbial derivatives, Libsen was found most effective considering mortality followed by Suspend 5SG and Ambush 1.8EC. The highest percentage of weight reduction was observed in Karanja oil (15%) and the lowest (14%) was in Mahogany oil. The highest

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percentage of weight reduction was obtained from Ambush 1.8EC (16%) and the lowest percentage of weight reduction was obtained from Suspend 5SG (13%). No. of eggs laid per female was the highest in mahogany oil (24) and lowest in Libsen (19). Percent of hatchability was highest in mahogany oil (21%) and lowest in Libsen (16%). Larval duration was the highest in mahogany oil (16 days) and the lowest was in Suspend 5SG (13 days). Pupal period highest in neem oil (7 days) and the lowest was in (6 days). The highest adult longevity (22 days) from the seeds treated with karanja oil. The lowest adult longevity (18 days) from seeds treated with Libsen. Thus, Karonja, Neem and Mahogany oil, Libsen, Ambush 1.8EC and Suspend 5SG were found effective against pulse beetle in storage. Therefore, these biorationals might be included in the development of IPM packages for the management of pulse beetle in the storage.

Keywords: Biorational insecticides; pulse beetle; storage; gram seed.

1. INTRODUCTION

Pulses serve as one of the main sources of protein and minerals as well as play a vital socio-economic role in the diet of common people of Bangladesh. Hence it has been considered as “poor man’s meat.” Pulses play a vital role in the diet of common people of Asian countries including Bangladesh. Pulses provide an inexpensive source of highly nutritious vegetables dietary protein (20-40%) [1]. Unfortunately, the average per head pulse consumption in Bangladesh is only 12g. As a result, the people of Bangladesh suffer from malnutrition. A large number of pulses are grown in Bangladesh such as lentil, gram, grasspea, mungbean, cowpea, etc. and the total production of pulse was 2,85,783 mectric tons from the area of 5,36,874 acres in 2014-2015 [2]. After harvesting, about 85% of the pulse growers in Bangladesh store pulses throughout the year in their houses [3]. Almost all pulse growers store the required quantity of pulse seeds in their house for growing next year. Unfortunately, in storage, pulses suffer enormous losses due to pest attack. Rahman (1971) reported 12% loss due to pulse beetle infestation in pulses stored in warehouse [4]. Three species of pulse beetle are reported to attack pulses in storage. These are *Callosobruchus chinensis*, *Callosobruchus maculates* and *Callosobruchus anails*. Among them, the pulse beetles *Callosobruchus chinensis* is the major pests in stored pulse [5,6,7]. Pulse beetle, *Callosobruchus chinensis* is a major economically important pest of all pulses and causes 40-50% losses of pulses in storage [8].

Management of this beetle is a challenge for growers because of hard to detect the initial damage caused by beetles and immature spend their whole life inside the seed. Different preventive and curative measures are practiced to control insect pest in field and in the storage. Uses of synthetic chemicals are major control measures for agricultural insect pests all over the world for both field and storage [9]. During storage for grain protection, liquid insecticides and gases in the form of phosphine and methyl bromide have been widely used as it is cost-effective and simple method [10,11,12,13].

Biorationals are Plant-derived materials which more readily biodegradable, less toxic to mammals, more selective in action, and retard the development of resistance. Hence, search for the alternative method of pulse beetle control utilizing some non-toxic, environment friendly and human health hazard free methods are being pursued now-a-days.

In the world, as many as 2400 plant species have been reported that have potential pesticidal properties and biological activity against a wide range of pests [14]. It was reported that when mixed with stored-grains, leaf, bark, seed powder or oil extracts of plants reduce oviposition rate and suppress adult emergence of bruchids, and also reduced seed damage [10,15,16]. Plant-based insecticides induce not only acute toxicity to pests but also deterrence and/or repellence which may contribute to overall efficacy against insect pests that cause great economic losses. Botanicals are desirable alternatives to synthetic chemical insecticides for controlling pests.

The undesirable and harmful effects of insecticides coupled with their high cost promoted to conduct the present experiment. Three botanicals such as Mahogany, Neem, Karonja oil and three microbial derivatives such as Libsen, Ambush 1.8EC and Suspend 5SG were evaluated for the effectiveness against pulse beetle, *C. chinensis* in storage under laboratory conditions. The present research was conducted to explore the effect of biorationals on
different biological parameters and managing of pulse beetle, *C. chinensis*.

2. MATERIALS AND METHODS

The present research work was conducted in the post-graduate laboratory, Department of Entomology, Bangladesh Agricultural University, (BAU), Mymensingh, using Completely Randomized Design (CRD) with three replications for each treatment during the period from February to March, 2018.

2.1 Collection of Gram Seed

The gram seeds were bought from the local market of BAU campus, Mymensingh. The seeds were properly dried and made completely free from insects, their eggs and microorganisms. Collected seeds were stored in a sealed container for further using in the experiment.

2.2 Source and Culture of Pulse Beetles

Gram seeds infested with Pulse beetles were collected from the Laboratory of Bangladesh Institute of Nuclear Agriculture (BINA) to maintain a laboratory culture under 27±2°C temperature and 75±5% relative humidity. The container was covered with nylon netting cloth for proper ventilation and kept in the laboratory. Then insects were allowed for free mating and oviposition for a maximum period of 10 days. The seeds with eggs were kept for emergence of new adults. Newly emerged beetles were used in different experiments.

2.3 Treatments of the Experiment

There were 7 treatments including one control that were applied against pulse beetles infesting gram in the storage condition Viz. Mahogany oil, Neem oil, Karanja oil with 0.5, 1.0, 1.5 ml/L of each, Suspend 5SG 0.5, 0.75, 1.0 gl/L, Libsen, Ambush 1.8 EC with 0.5, 0.75, 1.0 ml/L of each.

2.4 Test Plant Materials

Mahogany (*Swietenia mahogoni* L.)- Meliaceae, is a large tropical tree with a semi evergreen growth habit. The oil of mahogany fruit has insecticidal, insecticidal, antiseptic properties. Neem (*Azadirachta indica* A Juss.)- Meliaceae Neem leaves are dried in India and placed in cupboards to prevent insects eating the clothes and also in tins where rice is stored. Neem oil is used for healthy hair, to improve liver function, and balance blood sugar levels. Karanja (*Pongamia pinnata* L Pierre)- Fabaceae, native in tropical and temperate Asia. It has a straight or crooked trunk, 50-8 centimetres in diameter with grey-brown bark. Juices from the plant, as well as the oil, are antiseptic and resistant to pests. Libsen (Trade name: Libsen 45 SC) both contact and stomach action along with translaminar movement properties. The mode of action of Libsen is via a neural mechanism. Libsen kills insects via hyper excitation of the insect nervous system. Ambush 1.8 EC (Trade name: Benten 1.8 EC), an insecticide as well as acaricide. It has the capacity of contact or stomach actions sometimes with local systemic activity. The compound stimulates the release of GABA from nerve endings and enhances the binding of GABA to receptor sites on the post-synaptic membrane. Suspend 5 SG (Trade name: XPERT 5 SG), Suspend 5SG is a new insecticide of Syngenta Crop Protection, with a new mechanism of action and a strong activity against Lepidoptera as well as with and a high selectivity on useful organisms.

2.5 Bioassay and Data Collection Procedures

2.5.1 Assessment of toxicity by direct method

About 30 g gram seeds and 15 pulse beetles were taken in Petri dishes previously washed with alcohol. There were three replications of each pesticide tested. By the help of spray machine, different doses of botanicals (neem, mahogoni and karanja oil) and microbial derivative pesticides (Ambush 1.8 EC, Libsen and Suspend 5SG) solutions were sprayed in the Petri dishes. Data on mortality of pulse beetle were recorded at 24, 48 and 72 hrs after treatment.

2.5.2 Assessment of toxicity by indirect method

By the help of spray machine, different doses of botanical (neem, mahogoni and karanja oil) and microbial derivative pesticides (Ambush 1.8 EC, Libsen and Suspend 5SG) were sprayed in the Petri dishes. There were three replications of each pesticide tested. Data on mortality of pulse beetle were recorded at 24, 48 and 72 hrs after treatment.
2.6 Calculation of Per cent Mortality

Number of dead and alive insects was regularly observed and percent mortality was calculated by using the following formula:

\[
\text{Percent mortality} = \frac{\text{No. of dead pulse beetle}}{\text{Total no. of released pulse beetle}} \times 100
\]

2.7 Per cent Weight Loss

Percent weight loss and percentage protection of seed weight over control were calculated with the following formula:

\[
\text{Percent weight loss} = \frac{\text{Weight loss per Petridish}}{\text{Initial weight of seed grains per Petridish}} \times 100
\]

Where,

\[
\text{Weight loss per Petridish} = (\text{Initial weight-final weight}) \text{ of grains per petridish}.
\]

\[
\text{Weight Protection Over Control} = \frac{\text{Weight loss in control} - \text{Weight loss in treatments}}{\text{Weight loss in control}} \times 100
\]

2.8 Data Collection on Adult Longevity and Fecundity of Pulse Beetle

Data on fecundity (no. of eggs laid, percent of hatchability), development (larval duration, pupal period) and adult longevity of pulse beetle were collected by the same bioassay procedure.

2.9 Statistical Analysis

All the recorded data were compiled and tabulated for statistical analysis. Analysis of variance was done with the help of a computer statistical package MSTAT. The mean differences among the treatments were adjudged with Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) when necessary.

3. RESULTS AND DISCUSSION

3.1 Efficacy of Some Selected Botanicals on the Mortality of Adult Pulse Beetle, *C. chinensis* L

The effect of different concentration of Neem oil, Mahogany oil and Karanja oil on the mortality of pulse beetle both by direct and indirect bioassay method is presented in Table 1. For neem oil, direct bioassay showed higher mortality. All the doses showed significant effect on the mortality of beetles in comparison to control. At 3 DAS and 1.50 ml/L concentration the highest mortality (77.78%) of beetles was found in neem oil and the lowest mortality was found in untreated control (17.78%) (Table 2). The present study on adult mortality was consistent with the results reported by Regmi and Dhoj [17]. They examined Neem oil at 1.50 ml/L caused the highest mortality of second (92.33%), third (95.23%) and fourth instar (76.19%) larvae. In case of indirect method of neem oil, the death of pulse beetle was comparatively lower than the direct bioassay method (Table 2). All the doses always showed significant effect on the mortality of beetles in comparison to control. At 3 DAS and 1.5 ml/L dose of Neem oil caused the highest mortality (33.33%) of beetles. Grainge and Ahmed (1988) observed that, under storage condition the botanicals like neem kernel powder (*Azadirachta indica*) was most effective against pulse beetle [14].

For mahogany oil in direct bioassay method the highest mortality (68.89%) was found in 1.50ml/L and the lowest mortality (17.78%) was in control at 3 DAS.

In case of indirect bioassay method, Mortality was increased with the treatment doses and time intervals. All the doses always showed similarly significant effect on the mortality of beetles up to 3 DAS and the highest mortality (33.33%) of beetle was found at 1.50 ml/L and the lowest mortality was in control (15.56%). Similar to neem oil, lower mortality was found in indirect bioassay method.

For karanja oil, significant effect on the mortality of the pulse beetle (Table 1). Mortality was increased with the higher doses of treatment and time. The highest (62.22%) and the lowest (15.56%) mortality of beetles were found at 3 DAS with 1.50 ml/L Karanja oil and control treatment, respectively. On the indirect method the highest (37.78%) and the lowest (11.11%) mortality of beetles were found at 3 DAS with 1.50 ml/L Karanja oil and control treatment, respectively. Similar to neem and mahogany oil, lower mortality was found in indirect bioassay method. The previous findings of Singh and Pandey, (2016) evaluated the efficacy karanja (*Pongamia pinnata*) oil against *C. chinensis* in green gram and found it was very effective for the protection of pulse beetle [18].
### Table 1. Effect of different doses neem oil, mahogany oil and Karanja oil on the mortality (%) of pulse beetle under lab condition

| Doses (ml/L) | Neem oil Direct bioassay method | Neem oil Indirect bioassay method | Mahogany oil Direct bioassay method | Mahogany oil Indirect bioassay method | Karanja oil Direct bioassay method | Karanja oil Indirect bioassay method |
|--------------|---------------------------------|----------------------------------|-------------------------------------|--------------------------------------|---------------------------------|-------------------------------------|
|              | 1DAS*                           | 2DAS                             | 3DAS                                | 1DAS                                 | 2DAS                             | 3DAS                                |
| 0.50         | 35.56b                          | 46.67b                           | 60.00b                              | 8.88b                                | 15.56b                           | 22.22b                              |
| 1.0          | 42.22b                          | 55.56ab                          | 64.44b                              | 17.78a                               | 24.44a                           | 31.11a                              |
| 1.50         | 51.11a                          | 62.22a                           | 77.78a                              | 24.44a                               | 33.33a                           | 44.44c                              |
| Control      | 4.44c                           | 13.33c                           | 17.78c                              | 4.44c                                | 11.11c                           | 17.78c                              |
| CV (%)       | 13.33                           | 12.50                            | 9.26                                | 30.98                                | 11.17                            | 9.46                                |
| Level of     | **                              | **                               | **                                  | **                                   | **                               | **                                  |

**Different letters in the same column indicate significant difference at 1% level of probability, DAS = Days after sowing**

### Table 2. Effect of different doses of Suspend 5SG, Libsen, Ambush 1.8EC on the mortality of pulse beetle under lab condition

| Doses | Suspend 5SG (gl/L) Direct bioassay method | Suspend 5SG (gl/L) Indirect bioassay method | Libsen (ml/L) Direct bioassay method | Libsen (ml/L) Indirect bioassay method | Ambush 1.8EC (ml/L) Direct bioassay method | Ambush 1.8EC (ml/L) Indirect bioassay method |
|-------|------------------------------------------|--------------------------------------------|------------------------------------|--------------------------------------|-------------------------------------------|---------------------------------------------|
|       | 1DAS                                    | 2DAS                                      | 3DAS                               | 1DAS                                 | 2DAS                                      | 3DAS                                        |
| 0.50  | 17.78b                                  | 31.11b                                    | 44.44c                             | 8.88c                                | 15.56c                                    | 22.22c                                      |
| 0.75  | 28.89a                                  | 33.33b                                    | 53.33b                             | 22.22b                               | 26.67b                                    | 35.56b                                      |
| 1.0   | 35.56a                                  | 42.22a                                    | 64.44a                             | 40.00a                               | 46.67a                                    | 55.56a                                      |
| Control | 4.44c                                  | 11.11c                                    | 20.00d                             | 8.88c                                | 15.56c                                    | 22.22c                                      |
| LSD   | 8.88                                    | 7.362                                     | 8.00                               | 8.59                                 | 10.17                                     | 7.36                                        |
| CV (%) | 20.51                                   | 12.52                                     | 8.79                               | 21.52                                | 19.92                                     | 11.44                                       |
| Level of | **                                      | **                                        | **                                 | **                                   | **                                        | **                                          |

**Different letters in the same column indicate significant difference at 1% level of probability, DAS = Days after sowing**
3.2 Efficacy of Some Selected Microbial Derivatives on the Mortality of Adult Pulse Beetle, C. chinensis L.

3.2.1 Effect of Suspend SSG on the mortality of adult pulse beetle

The effect of different concentration of Suspend 5 SG on the mortality of pulse beetle both by direct and indirect bioassay method is presented in Table 2. Suspend 5 sa showed significant mortality effect on the pulse beetle in comparison to control at 3 DAS of spraying with the doses of 0.50, 0.75 and 1.0g (Table 2). Mortality was increased with the increasing doses of Suspend 5 sa and time intervals. At 3 DAS and 1.0g/L concentration the highest mortality (64.44%) of beetles was found in Suspend 5 sa and the lowest mortality was found in untreated control (20.00%). In indirect bioassay method at 3 DAS and 1.0g/L concentration the highest mortality (55.56%) of beetles was found in Suspend 5 SG and the lowest mortality was found in untreated control (17.78%).

3.2.2 Effect of Libsen on the mortality of adult pulse beetle

All the doses showed significant effect on the mortality of beetles in comparison to control. At 3 DAS and 1.0ml/L dose the highest mortality (73.33%) of beetles was found in Libsen and the lowest mortality was found in control (22.22%) (Table 2).

On the indirect bioassay method of Libsen, the death of pulse beetle was lower than the direct method and has significant effect on the mortality of beetles in comparison to control. At 3DAS and 1.0 ml/L dose the highest mortality (55.56%) of beetles was observed in Libsen and the lowest mortality was found in control (22.22%) (Table 2). Our observations are in consistent with the previous findings of Thompson et al. (2000) who showed that different formulations of Libsen (liquid or dust) could be effective against several stored-product pests including pulse beetle especially on resistant strains of the pests [19]. Hameed et al. (2012) reported that Libsen was the best for controlling stored pests [20]. The present report indicated the toxicity of Libsen to pulse beetle, C. chinensis and also supported by the reports of Vayias et al. (2009) and Hertlein et al. (2011) who reported the toxicity of Libsen to coleopteran stored-grain pests [21,22].

3.2.3 Effect of Ambush 1.8 EC on the mortality of adult pulse beetle

Ambush 1.8 ECIs one of the effective biorationals which was used to control pulse beetle. Spraying at different doses viz 0.50, 0.75 and 1.0 ml/L Ambush 1.8 ECshowed significant effect on the mortality of the pulse beetle (Table 2). Mortality was increased with the higher doses of treatment and time. The highest (55.56%) and the lowest (17.78%) mortality of beetles were found at 3 DAS with 1.0 ml/L Ambush 1.8 ECand control treatment, respectively.

Significantly higher effect on the mortality of pulse beetle than the control was found at 3 DAS with 0.50, 0.75 and 1.0 ml/L of Ambush 1.8 ECspraying (Table 2) in case of indirect method. At 3 DAS and 1.0 ml/L concentration the highest mortality (48.89%) of beetles was found in Ambush 1.8 ECand the lowest mortality was found in untreated control (15.56%). Abd El-Razik and Zayed (2014) reported LC50 of ambush 1.8EC; 19964.04 ppm after 9 days post treatment against Callosobruchus maculatus (F.) adults [23].

3.3 Percentage Weight Loss of Gram Seeds Caused by C. Chinensis under Different Biorational Insecticides Treatment

It was indicated that weight reduction of seeds caused by pulse beetle was reduced with the increasing of doses of botanical insecticides. At 1.5ml/L the highest per centage weight reduction was observed in Karanja oil (15.25%) and the lowest (14.88%) was in Mahogany oil followed by Neem oil (15.12%).

3.4 Percentage Weight Loss of Gram Seeds Caused by C. Chinensis under Different Microbial Derivatives

The effect of different concentrations of different microbial derivative pesticides on seed weight loss due to infestation of pulse beetle was varied non-significantly (Table 3). But it was indicated that weight reduction of seeds caused by pulse beetle was reduced with the increasing of doses of biorational insecticides. The highest per centage of weight reduction was obtained from the 0.50ml/L dose of Ambush 1.8 EC(16.25) treated seeds and the lowest per centage of weight reduction was obtained from 0.50ml/L dose of Suspend 5 SG (13.66).
3.5 Effect of Some Selected Biorational Insecticides and Botanicals on the Longevity and Fecundity of Pulse Beetle, *C. chinensis*

In addition to evaluating the toxicity of selected biorational insecticides against pulse beetle, *C. chinensis*, the longevity and fecundity of pulse beetle were also observed and the results are presented in Table 4. The effect of different concentrations of biorational insecticides and botanicals on number of eggs laid by female of pulse beetle was statistically significant. From the results it was clearly observed that no. of eggs laid per female was the highest in mahogany oil (24.00/female) and the lowest in Libsen (19.33/female). Per cent of hatchability of *C. chinensis* treated with different biorational insecticides and botanicals varied non-significantly (Table 4). Per cent of hatchability was the highest in mahogany oil (21.00) and the lowest in Libsen (16.33) treated seeds. The effect of different concentrations of biorational insecticides on the larval duration was statistically significant. From the results it was clearly observed that larval duration was the highest in mahogany oil (16.00) and the lowest in Suspend 5SG (13.67). On the other hand, pupal period was the highest in neem oil (7.66) and the lowest in Libsen (6.33) which was found statistically non-significant. A non-significant difference was found in adult longevity from treated seeds. The total number of adults emerged from gram seeds treated with different concentration of biorational insecticides are presented in Table 4. Among the treated seeds, the highest number of adult longevity (22.00) from the seeds treated with karanja oil. The lowest number of adult longevity (18.67) from seeds treated Libsen.

Table 3. Per centage weight reduction of gram seeds caused by *C. chinensis* treated with different microbial derivatives

| Botanicals | Neem Weight reduction (%) | Mahogany | Karanja |
|------------|---------------------------|----------|---------|
| 0.50 ml/L  | 14.64a                    | 13.48a   | 14.27a  |
| 1.0 ml/L   | 14.71a                    | 13.93a   | 14.52a  |
| 1.5 ml/L   | 15.12a                    | 14.88a   | 15.25a  |
| Control    | 14.29a                    | 14.29a   | 14.29a  |
| Level of significance | NS | NS | NS |
| CV (%)     | 1.94                      | 10.02    | 4.91    |

| Biorationals | Ambush 1.8EC | Suspend 5SG | Libsen |
|--------------|--------------|-------------|--------|
| 0.50 ml/L    | 16.25a       | 13.66a      | 13.93a |
| 0.75 ml/L    | 15.68a       | 14.53a      | 14.37a |
| 1.0 ml/L     | 14.73a       | 14.95a      | 14.31a |
| Control      | 16.22a       | 15.25a      | 14.29a |
| Level of significance | NS | NS | NS |
| CV (%)       | 5.95         | 7.01        | 8.38   |

*Different letters in the same column indicate significant difference at 1% level of probability*

Table 4. Effect of some selected bio rational insecticides and botanicals on the longevity and fecundity of pulse beetle, *C. chinensis*

| Bio rationals | Doses (ml/L) | No of eggs laid/female | Per cent of hatchability | Larval duration (days) | Pupal period (days) | Adult longevity (days) |
|---------------|--------------|------------------------|--------------------------|------------------------|-------------------|------------------------|
| Ambush 1.8EC  | 1.0          | 20.00bc                | 18.33ab                  | 15.00c                 | 6.333a            | 19.33bc                |
| Suspend 5SG   | 1.0 g/L      | 22.33ab                | 20.00a                   | 13.67c                 | 7.000a            | 19.33bc                |
| Libsen        | 1.0          | 19.33c                 | 16.33b                  | 15.67ab                | 6.667a            | 18.67c                 |
| Mahogany oil  | 1.5          | 24.00a                 | 21.00a                  | 16.00ab                | 6.667a            | 20.67abc               |
| Karanja oil   | 1.5          | 22.67ab                | 19.00ab                 | 14.67bc                | 7.333a            | 22.00a                 |
| Neem oil      | 1.5          | 23.00ab                | 20.00a                  | 14.00c                 | 7.667a            | 19.33bc                |
| LSD           | 2.808        | 2.965                  | 1.427                    | 1.537                  | 2.377             |                       |
| CV (%)        | 7.16         | 8.68                   | 5.31                     | 12.42                  | 6.63              |                       |

*Level of Significance: * NS **

*Different letters in the same column indicate significant difference at 1% level of probability*
4. CONCLUSION

From the above discussion it can be conclude that, Karanja, Neem and Mahogany oil, Libsen, Ambush 1.8 EC and Suspend 5SG were found effective against pulse beetle in storage. Therefore, these biorationals might be included in the development of IPM packages for the management of pulse beetle in the storage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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