



RESEARCH ARTICLE

EFFICACY OF BOTANICAL DUST AGAINST PULSE BEETLE OF BLACK GRAM (*Vigna mungo* L.)Shanti Acharya^a, Srijana Shrestha^a, Ankit Ojha^b^aFaculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, 44200, Bagmati Province, Nepal^bAssistant Professor, Department of Genetics and Plant Breeding, Agriculture and Forestry University Rampur, Chitwan, 44200, Bagmati Province, Nepal^{*}Corresponding Author Email: acharya1998shanti@gmail.com

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ABSTRACT

The study aims to devise suitable and eco-friendly control measures for pulse beetle. For this, the experiment was carried out in Central Agricultural Laboratory, Hariharbhawan, Lalitpur from April 2020- June 2020. The experiment was laid out in a Completely Randomized Design with four replication and six treatments (Xanthoxylum armatum DC fruit dust@ 50gm kg⁻¹; Acorus calamus L. rhizome dust@ 50 gm kg⁻¹; Curcuma longa L. rhizome dust@ 50 gm kg⁻¹; Azadirachta indica A. Juss. leaf dust@50gm kg⁻¹; malathion dust@ 1gm kg⁻¹ and control). Out of four botanical dust, bojho rhizome was found more effective than Malathion (positive check) with minimum insect build-up (0.00) and minimum loss (0.00%). It was effective in maintaining higher germination (92.5 percent) and lower moisture content (11.25). X. armatum fruit dust and A. indica leaf dust were found ineffective to cause adult mortality (56.25 and 59.37) in black gram. Thus, botanical materials such as A. calamus rhizome dust has been found as effective safe alternatives for the management of C. chinensis L. in the storage of black gram.

KEYWORDS

Experiment, bojho rhizome, eco-friendly, effective, insect

1. INTRODUCTION

Pulses are ideal food grains for crop intensification, diversification, and sustainable farming systems; soil nitrogen economy, amenability to multiple and intercropping, and adaptability to marginal lands. Considering that they are the most affordable source of proteins, vitamins, energy, and minerals, pulses are crucial parts of our daily diet. Pulses are important for both sustainable agriculture and nutritional security (Arya et al., 2002).

Pulses are in general the most important type of food after cereals in any part of the world, especially in Southeast Asia where animal protein is scarce; they provide a large proportion of the protein requirement (COPR, 1981). In Nepal, grain legumes collectively rank fourth in terms of area and production after rice, maize, and wheat. They were grown in an area of 331.740 thousand hectares (ha) and with a total production of 381,987 metric tons (MT) during 2018/19 (MOAD, 2020).

Black gram (*Vigna mungo*) is one of the principal pulses and commonly used leguminous crop in hills because of its significant role in human as well as animal nutrition. Black gram production in Nepal had an average yield of 848 kilograms per hectare in the 2018/19 growing season, and was the third highest produced pulse in Nepal, only being surpassed by lentil and soya bean production (MOAD, 2020).

Among various agricultural constraints, insect pests are major bottlenecks in realizing higher yields. Almost all the insect pests of stored grains have a remarkably high rate of multiplication and within one season, they may destroy the grain and contaminate the rest with undesirable color, odor, and flavor. The annual grain loss in storage due to insects is about 15% (Joshi et al., 1991). The bruchids are serious under storage. The damage in some pulses is so serious that whole of the seed material is eaten and only seed coats with empty cavities are left behind (Vir and Jindal, 1981). It also has a great impact on seed germination and moisture content and hampers

the nutritional quality as well. The development of a single larva in a pulse grain can lead to 8 to 22 percent weight loss and they are capable of destroying all grains by causing up to 100 percent losses within a few months of storage (Augustine and Balikai, 2019).

Keeping in view the high cost of treatment with recommended insecticides and harmful residues, several alternatives have been evaluated including the use of plant products like Timur (*Xythoxylum armatum*), sweet flag (*Acorus calamus*), turmeric (*Curcuma domestica*), etc. Botanicals are Generally Recognized as Safe (GRAS) approved by Food and Drug Administration (FDA) and Environment Protection Agency (EPA) in the USA for food consumption (Kedia et al., 2015). Moreover, chemical-based control measures are likely to be effective only in the short run. Thus, there is an urgent need to develop safe alternatives that have the potential to replace the toxic fumigants, yet are simple and convenient to use (Shaaya et al., 1997).

This study aims to solve the problem of Nepalese farmers at their level, using the locally available products which increase their income and don't hamper the quality of black gram and their quality. This study intends to evaluate the efficacy of different botanical dust for the management of pulse beetle in black gram by assessing their effect on seed quality parameters of black gram infested by pulse beetle and by testing the mortality and survival effects of botanical dust on pulse beetle infesting black gram. This study also focuses on identifying the effective eco-friendly control measure of pulse beetle in stored black gram seeds.

2. MATERIALS AND METHODS

2.1 Site of Study

The experiment was carried out at the Central Agricultural Laboratory, Hariharbhawan, Lalitpur, Nepal (Figure 1). Its geographical coordinates are 27° 40' 43" North, 085° 18' 58" East.

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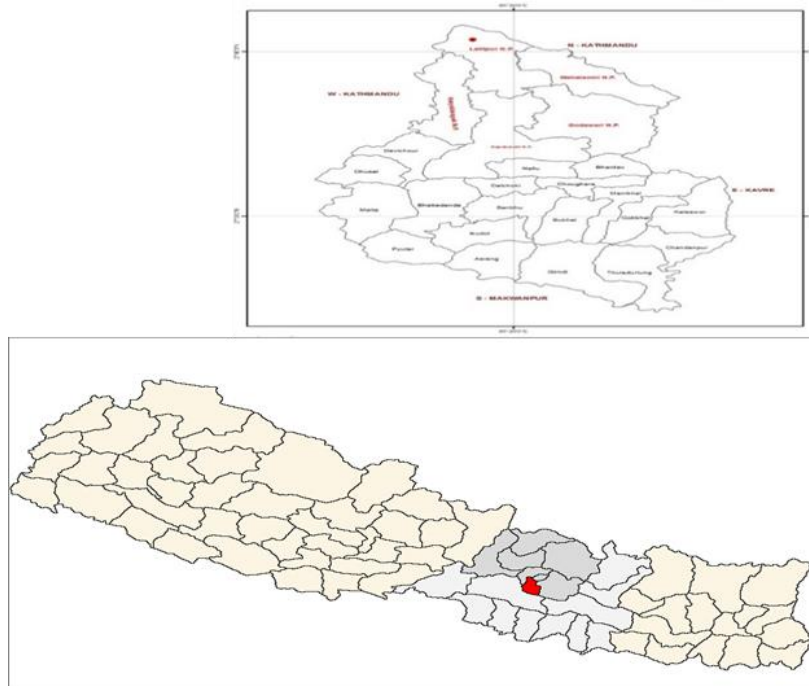


Figure 1: Map of Nepal showing Lalitpur District
Source: http://lgcdp.gov.np/GIS_district?page=4

2.2 Maintenance of Insect Culture

Live insect specimens of *C. chinensis* were collected from insect-infested stores. The insect was confirmed by entomologists. For maintenance of insect culture, mass rearing was performed in the bulk of black gram at room temperature. The culture was produced as a pure culture for the inoculation of each experiment.

2.3 Preparation of Seed

The seed was bought from the market, then fumigated using celphos for 72 hours, then washed and sun-dried. Initial moisture content, germination percentage, and 1000 seed weight were recorded. Table 1 shows the six treatments; botanical materials used along with their doses for the management of bruchids.

Table 1: Botanical Materials Used in The Management of Bruchids (Lalitpur, 2020)

Treatments	Dose	
T1	Timur (<i>Xanthoxylum armatum</i> DC) fruit dust	50g/kg of seed
T2	Bojho (<i>Acorus calamus</i> L.) rhizome dust	50g/kg of seed
T3	Neem (<i>Azadirachta indica</i> A. Juss.) leaf dust	50g/kg of seed
T4	Turmeric (<i>Curcuma longa</i> L.) rhizome dust	50g/kg of seed
T5	Malathion dust (check)	1g/kg of seed
T6	Control	

2.4 Preparation of Botanical Powders

Fresh rhizomes of Bojho and Turmeric, fresh green leaves of Neem, and fruits of Timur were collected from the local market, washed, and air-dried in the shade for 4 days. Dried leaves, rhizomes, and seeds of respective plants were ground separately to a fine powder using a grinder.

2.5 Application of Treatments

The prepared botanical powders were uniformly mixed with healthy seeds at the dose of 50g / kg of seed for each treatment. The concentration of Malathion was 1g / kg of seed. 100g seeds were drawn from each treatment and stored in plastic jars with their lids covered by muslin cloth. The layout of the experiment can be seen in Table 2.

No. of replication: 4

Design: Complete Randomized Design

2.6 Observation and Recording Of Data

Count of total dead adults in each experiment unit at 24 hours, 48 hours, 72 hours, 96 hours, and 120 hours after treatment of adult bruchids were taken. The total no. of eggs laid in each experimental unit was counted after the death of all the insects kept. Total live and dead adults emerged at 65 days of storage {ignoring dead and live adults in hole} were counted. Germination percent of the seeds were taken before the experimental setup and also at the last of the experiment using the blotting paper method. For the assessment of moisture content, the oven-dried method was used and the moisture content of black gram grains was measured before the experimental setup and also at last. For the assessment of percent weight loss, a 1000-grain sample was taken randomly at the final recording followed by counting of damaged and undamaged grains. The weight of damaged and undamaged grains was recorded with the help of electronic balance. Also, 1000 seed weights were taken using electronic balance initially as well as at last from each treatment and replication.

Table 2: Layout of the Experiment (Lalitpur, 2020)

Replication 1	Replication 2	Replication 3	Replication 4
R ₁ T ₁	R ₂ T ₁	R ₃ T ₁	R ₄ T ₁
R ₁ T ₂	R ₂ T ₂	R ₃ T ₂	R ₄ T ₂
R ₁ T ₃	R ₂ T ₃	R ₃ T ₃	R ₄ T ₃
R ₁ T ₄	R ₂ T ₄	R ₃ T ₄	R ₄ T ₄
R ₁ T ₅	R ₂ T ₅	R ₃ T ₅	R ₄ T ₅
R ₁ T ₆	R ₂ T ₆	R ₃ T ₆	R ₄ T ₆

Storage duration: 65 days

Storage structure: Plastic Jar

Grain in each jar: 100 grams

No. of Insects per Jar: 8 newly emerged adults (in the assumption of a 1:1 male-female ratio)

2.6.1 Analysis of Germination Percent

To determine the viability of the seeds, a random sample of 25 grains of each treatment and replication were taken. Blotting papers were soaked in water and the grains were placed on the paper. Each paper was placed in an oven at 25°C until germination of the seed. Finally, all the germinated seeds were counted and the germination percentage was calculated using the following equation as followed by Baral et.al. (2002):

$$\text{Germination Percentage} = \frac{\text{Number of germinated grains}}{\text{Total number of grain}} \times 100$$

2.6.2 Assessment of Loss Percentage

The assessment was done simply using the count and weight method as adopted by Joost et al. (1996). For this, the number and weight of damaged and undamaged grains of a composite sample of 1000 grains were taken from each treatment and replication at the final observation. The percentage weight losses were calculated using the following equation:

$$\text{Loss Assessment} = \frac{(W_u \times Nd) - (W_d \times Nu)}{W_u \times (Nd + Nu)} \times 100$$

Where,

W_u = weight of undamaged grains

N_u = number of undamaged grains

W_d = weight of damaged grains

N_d = number of damaged grains

2.6.3 Analysis of Moisture by An Oven-Dried Method

The oven-dry method was adopted in the experiment. For this method, 25 grains of black gram were taken from each treatment and replication at random. The samples were coarsely crushed. The weight of Petri plates (M1) was recorded. After that, each sample was put on a well-labeled Petri plate. Initial weights were recorded with the help of an electric weighing machine (M2) and then all the plates were kept in an oven for 1 hour. The weighing was recorded after 1 hour (M3). At last, the moisture was calculated using the following mathematical formula:

$$\text{Moisture percentage} = \frac{(M_2 - M_3)}{(M_2 - M_1)} \times 100$$

2.7 Data Analysis

Microsoft Excel and R-studio packages were used for arranging the recorded biological parameters and for the analysis of variance (Table 3) and other data analysis.

Table 3: ANOVA Statistical Analysis

Source of Variation (SOV)	Degree of freedom (df)	Sum of Squares (SS)	Mean Sum of Squares (MSS)	Calculated F-value	Tabulated F value	
					5%	1%
Treatment (Tr)	5	TrSS	TrSS/5	TrMSS/EMSS	2.9	4.56
Error (E)	18	ESS	ESS/18			
Total (T)	23	TSS	TSS/23			

3. RESULTS

Maximum death occurred in grain treated with Bojho rhizome powder and Malathion dust (both 100.00%). This was found significantly different ($P=0.000$) compared to grain treated with Turmeric rhizome powder, Neem powder, Timur fruit powder, and Control (Table 4).

Table 4: Effect of Botanical Dust on Adult Mortality (%) of Pulse Beetle in Black Gram 5 DAT (Lalitpur, 2020)

Treatments	Adult mortality (%)
Timur fruit dust	56.25 ^b
Bojho rhizome dust	100.00 ^a
Neem leaf dust	59.37 ^b
Turmeric rhizome dust	62.50 ^b
Malathion (check)	100.00 ^a
Control	43.75 ^b
LSD (0.05)	19.93
SEm (\pm)	6.71
C.V. (%)	19.08
Mean	70.31

Values with the same letter in the given column are not significantly different by LSD 0.05 level.

The oviposition was least on Bojho rhizome powder (0.00) followed by Malathion (12.50) and Turmeric Rhizome powder (35.25) and was

maximum on control (Table 5). The difference was significant with a p-value of 0.001.

Table 5: Effect of Selected Botanical Dust on Oviposition of Pulse Beetle on The Black Gram (Lalitpur, 2020)

Treatments	Number of eggs
Timur fruit dust	72.0 (8.34) ^a
Bojho rhizome dust	0.00 (0.00) ^d
Neem leaf dust	67.0 (8.09) ^a
Turmeric rhizome dust	35.25 (5.85) ^b
Malathion (check)	12.50 (3.46) ^c
Control	85.0 (9.18) ^a
LSD (0.05)	1.70
SEm (\pm)	0.57
C.V. (%)	19.70
Mean	5.82

The value in parenthesis indicates square root transformed value and values with the same letter in the given column are not significantly different by LSD 0.05 level.

Similarly, the emergence of beetle was highest in control (36) which was significantly different from the rest ($P=0.001$) as presented in Table 6 and the least emergence was found in Bojho rhizome dust (0.00) followed by Malathion (2).

Table 6: Effect of Selected Botanical Dust on The Emergence of Pulse Beetle in Black Gram (Lalitpur, 2020)

Treatments	Number of adults emerged	Adult Emergence (%)
Timur fruit dust	13.00 (3.55) ^b	18.06
Bojho rhizome dust	0.00 (0.00) ^d	0.00
Neem leaf dust	12.00 (3.42) ^b	17.91
Turmeric rhizome dust	10.00 (3.01) ^b	28.36
Malathion (check)	2.00 (1.21) ^c	16
Control	36.00 (5.97) ^a	42
LSD (0.05)	0.521	
SEm (\pm)	0.36	
C.V. (%)	25.19	
Mean	2.86	

The value in parenthesis indicates square root transformed value and values with the same letter in the given column are not significantly different by LSD 0.05 level.

Figure 2 shows that the oviposition and emergence of pulse beetle on the black gram is highest in control whereas none in black gram treated with bojho rhizome dust.

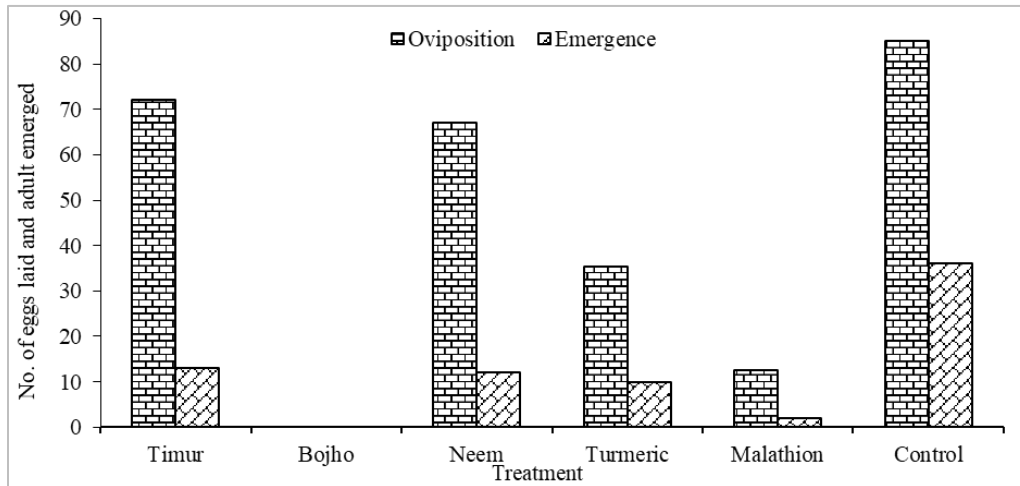


Figure 2: Effect of selected botanical dust on oviposition and emergence of pulse beetle on the black gram (Lalitpur, 2020)

Table 7 shows that the black gram seeds treated with bojho rhizome dust were completely safe from insect attack and significantly different ($P=0.001$) compared to the rest of the treatments. The highest kernel damage was recorded in control (1.13%).

Treatments	Seed loss (%)
Timur fruit dust	0.96 ^a
Bojho rhizome dust	0.00 ^b
Neem leaf dust	0.72 ^a
Turmeric rhizome dust	0.83 ^a
Malathion (check)	0.70 ^a
Control	1.13 ^a
LSD (0.05)	0.50
SEm(±)	0.17
C.V. (%)	46.02
Mean	0.72

Values with the same letter in the given column are not significantly different by LSD 0.05 level.

Table 8 reveals that the highest grain loss was found in control (4.1%) and the least loss was observed in seeds treated with bojho rhizome dust (0.52%). With this result, bojho powder was found more effective than the chemical check (Malathion) ($p=0.000$)

There was a significant change in 1000 seed weight of black gram (Table 9). The magnitude of change was significantly higher ($p=0.019$) in control followed by Timur fruit powder. The change was lower in bojho rhizome powder and Malathion. The reduction of 5.74% of 1000 seed weight was seen in control whereas in bojho rhizome powder it was only 0.45%. It is evident that insect population was positively correlated with percentage grain damage, percent weight loss, and moisture content, however, it is inversely proportional to 1000- seed weight and germination percentage of seed.

Maximum germination (9.68%) was reduced in control and it was significant ($p=0.000$) over the rest of the treatments (Table 10). Whereas, minimum germination reduction was found on bojho rhizome treatment (0.54%) followed by Malathion (4.30%).

Treatments	Initial seed weight (g)	Final seed weight (g)	Reduction (%)
Timur fruit dust	100	97.66 ^b	2.34
Bojho rhizome dust	100	99.47 ^a	0.53
Neem leaf dust	100	98.18 ^{ab}	1.82
Turmeric rhizome dust	100	98.33 ^{ab}	1.67
Malathion (check)	100	98.61 ^{ab}	1.38
Control	100	95.89 ^c	4.10
LSD (0.05)		1.33	
SEm(±)		0.449	
C.V. (%)		0.92	
Mean		98.02	

Values with the same letter in the given column are not significantly different by LSD 0.05 level.

Treatment materials	Initial weight (g)	Final 1000 seed weight (g)	Reduction (%)
Timur fruit dust	49.30	47.05 ^c	4.56
Bojho rhizome dust	49.30	49.07 ^a	0.45
Neem leaf dust	49.30	47.68 ^{bc}	3.28
Turmeric rhizome dust	49.30	48.55 ^{ab}	1.52
Malathion (check)	49.30	49.05 ^a	0.51
Control	49.30	46.47 ^c	5.74
LSD (0.05)		1.235	
SEm(±)		0.41	
C.V. (%)		1.73	
Mean		47.97	

Values with the same letter in the given column are not significantly different by LSD 0.05 level.

Table 10: Effect of Selected Botanical Dust Germination (%) of Black Gram Due to Pulse Beetle 65 DAT (Lalitpur, 2020)

Treatments	Initial germination %	Final germination %	Reduction (%)
Timur fruit dust	93	85.00 ^c	8.60
Bojho rhizome dust	93	92.50 ^a	0.54
Neem leaf dust	93	85.00 ^c	8.60
Turmeric rhizome dust	93	88.00 ^b	5.38
Malathion (check)	93	89.00 ^b	4.30
Control	93	84.00 ^c	9.68
LSD (0.05)		2.643	
SEm(±)		0.890	
C.V. (%)		2.040	
Mean		87.25	

Values with the same letter in the given column are not significantly different by LSD 0.05 level.

Table 11: Effect of Selected Botanical Dust on Moisture Content (%) of Black Gram (Lalitpur, 2020)

Treatments	Initial germination %	Final germination %	Reduction (%)
Timur fruit dust	93	85.00 ^c	8.60
Bojho rhizome dust	93	92.50 ^a	0.54
Neem leaf dust	93	85.00 ^c	8.60
Turmeric rhizome dust	93	88.00 ^b	5.38
Malathion (check)	93	89.00 ^b	4.30
Control	93	84.00 ^c	9.68
LSD (0.05)		2.643	
SEm(±)		0.890	
C.V. (%)		2.040	
Mean		87.25	

Values with the same letter in the given column are not significantly different by LSD 0.05 level.

There was a significant difference ($p=0.000$) in moisture content (Table 11). The increment in moisture was highest in control (5.93) and was significantly different compared to the rest. The rest of the treatments were at par.

Table 12 showed a total population of bruchid was found positively correlated with grain damage (0.644**), and grain moisture content (0.88**). It showed an inverse relationship between the total population of bruchid with that of total grain loss (-0.85**) and germination% (-0.74**). Likewise, a negative correlation was found between grain damage

% with germination (-0.85**) and grain loss (-0.80**), but a positive correlation (0.51*) was found in damage % with moisture content (0.51**). The grain loss had a positive correlation with germination (0.78*) and a negative correlation with moisture (-0.80**). Germination% and moisture % were negatively correlated (-0.52**).

Figure 3 shows that the grain damage (%), total grain weight loss (%), reduction in germination (%), and increment in moisture (%) are lowest in black gram treated with bojho rhizome dust whereas highest in case of control.

Table 12: Effect of Selected Botanical Dust to Bruchid on Black Gram After 65 Days of Storage (Lalitpur, 2020)

Parameters	Damage%	Grain loss	Moisture%	Germination%
Emergence	0.644**	-0.85**	0.88**	-0.74**
Damage%	-	-	-	-
Grain loss	-0.80**	-	-	-
Moisture%	0.51**	-0.80**	-	-
Germination%	-0.85**	0.78*	-0.52**	-

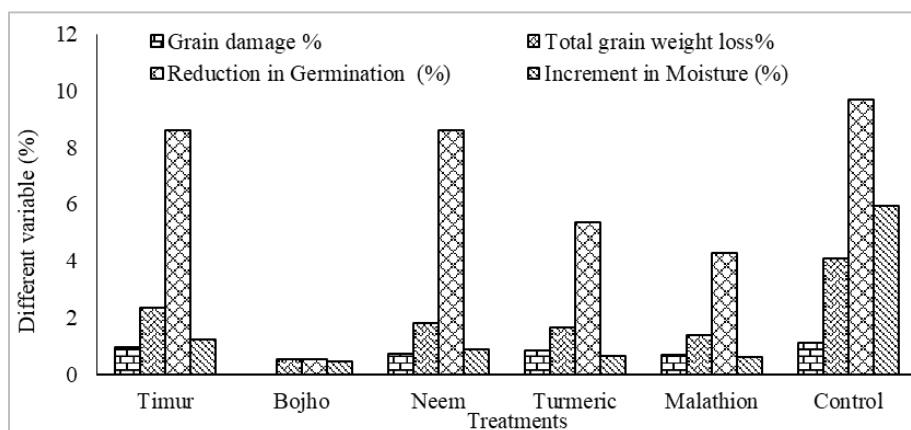


Figure 3: Relationship among different parameters in the management of bruchids with selected botanicals (Lalitpur, 2020)

4. DISCUSSION

Black gram seeds treated with different botanical dust materials were

significantly different and effective against *C. chinensis*. Bojho rhizome dust was found most effective in terms of less pest build-up (0.00) and grain loss (0%). It has also given the highest germination (92.5%) over

Malathion. A similar result was reported by Baral (2002) and Regmi and GC (2011). A study reported that 82.96% mortality of pulse beetle; 0.00% weight loss, and 2.19% moisture increment after two generations of pulse beetle using 30% *A. calamus* rhizome dust and was found effective than malathion to control pulse beetle (Regmi and GC, 2011). Minimum damage in seeds treated with botanical dust is because of oviposition deterrent and adult emergence inhibition activity of botanical extracts and also due to physical properties causing changes in surface tension and oxygen tension within the eggs (Abdullahi et al., 2011). A study in Chitwan reported 4.25% seed damage in control and 0.00% in *A. calamus* L. (50g/kg) after 35 days of storage of cowpea infested with bruchids (Baral, 2002). The difference in value might be due to the differential preference of bruchids for different legumes and also the experiment was carried out in different ecological regions (Varma and Anandhi, 2010). Baral (2002) reported a 3% increment in moisture and 0.85% reduction in germination percentage in *A. calamus* L. treated seeds against a 40% increment in moisture and a 20% reduction in germination in control due to bruchids after 105 days storage of cowpea.

Similarly, a study reported the use of several botanicals like *Eucalyptus globules* L., and *Ipomea seiparia* Roxb. @ 3% dose protected black gram from pulse beetle without adversely affecting the germination for three months. *A. calamus*, L. (10g/kg) treated maize recorded the lowest seed damage caused by weevil among all treatments viz. *A. indica* A. Juss (seed dust), *Z. alatum* L. (fruit dust), *Justicia adhatoda* L. (leaf dust), *M. azedarach* L. (seed dust) and *A. vulgaris* L. (leaf dust) at six months after treatment (Tiwari et al., 2018; Agarwal, 2017). The dust of turmeric rhizome dust and neem leaf powder was also found to be promising botanical pesticides, although they were not as effective as bojho rhizome dust and malathion. They have induced low pest build-up (10 and 12) and grain loss (1.67% and 1.82%) as compared to the further two treatments and the germination is relatively better (88.0% and 85.0% respectively). Singh et al. (1994) reported that vegetable oil is active against eggs and larvae and makes them unable to oviposit or cause mortality of the adult bruchids. Only a small amount of oil is needed to preserve the grains for months (1-5 ml oil kg⁻¹ of grains). Paneru et al. (1997) reported that powder of bojho rhizome was found as superior over fumigants (aluminium phosphide) to control *Sitophilus* spp. in wheat and maize grains.

Moreover, bojho does not cause any negative effects on human health and the environment. Therefore, it is regarded as a more suitable storage pesticide than other recommended fumigants (Panthee, 1997). *A. calamus* was found even more effective than malathion in controlling *S. oryzae* in wheat seed storage (Panthee, 1997). *A. calamus* is an herb, which does not have any negative effect on human health and the environment and is available easily, which is likely to be accepted by the farmers (Panthee, 1997). A compound *B*-asarone with an insecticidal activity was found in *A. calamus* L. rhizome thought to be responsible for the mortality of weevils (Tiwari et al., 2018) due to the destruction of physical barriers and spiracle blockage (Selva et al., 2019). Baral (2002) found that 17% of grain damage by *A. indica* leaf dust treated but 0% in bojho treated grains in 70 days against *C. maculatus* (F.). Here, the damage percentage in our experiment is 0.7223% in *A. indica* leaf dust treated grains. This may be due to unfavorable weather conditions for *C. chinensis* this year as well as due to the varietal preference of the beetle.

5. CONCLUSION

The study found that out of the four different botanical dust, bojho rhizome dust absorbs less moisture and has caused minimum pest build-up during the entire period of storage leading to reduced grain damage and grain loss thus retaining maximum grain germination which is seen as more effective than malathion. The mortality was highest in bojho rhizome dust. Similarly, oviposition was also significantly reduced in bojho rhizome dust. Thus, botanical materials such as *A. calamus* rhizome dust @50g kg⁻¹ can be explored as excellent alternatives over poisonous pesticides for the management of *C. chinensis* L. in the storage of black gram. These practices may reduce the need for chemical pesticides and botanical pesticides can be promoted as eco-friendly measures for the management of bruchid in black gram and other related pulses. This botanical pesticide is hoped to reduce the development of pest resistance against Malathion and the detrimental effects of chemical fumigants used in storage structures. Similarly, the botanical materials which reduce pest infestation below than economic injury level could be promoted as an important component of integrated pest management (IPM).

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