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EVALUATION OF BOTANICAL GRAIN PROTECTANTS AGAINST PULSE BEETLE (CALLOSOBRUCHUS MACULATUS FABRICIUS)

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ABSTRACT The investigations were conducted to check the efficacy of different grain protectants against pulse beetle (*C. maculatus*) on stored pigeonpea. The results revealed that the black pepper (25 g/kg of seed) powder provided complete protection of pigeonpea seeds from *C. maculatus*. It was the most effective treatment which recorded less egg laying, adult emergence, weight loss, seed damage and high feeding index next best treatment was clove powder and neem leaf powder. Even after two months of treatments, none of the botanical powders had a negative impact on the germination of pigeonpea seeds. *Keywords*: Pulse beetle, *Callosobruchus maculatus*, Pulse, Pigeonpea, Grain protectant.

Introduction

Pulses are "Wonderful gift of nature" that have a vital role in the Indian economy and diet both. It is an important segment of Indian agriculture after cereals and oilseeds. Pulses constitute the key source of dietary protein and vitamins predominantly for vegetarians around the world. Also preserves soil fertility via biological nitrogen fixation and hence plays an important role in sustainable agriculture. Pigeonpea (*Cajanus cajan* Linn. Millsp.) is the second most significant pulse crop after chickpea in India. Locally, it is referred to as arhar, tur, or red gram. The pigeonpea plant originated in India. It is cultivated in tropic and sub-tropic areas around the world.

Both qualitative and quantitative losses in stored pigeonpea seed can be caused by biotic (insects, mites, rodents, etc.) and abiotic (relative humidity, temperature, grain moisture, etc.) factors. Among different pests, pulse beetle, *Callosobruchus* spp. cause huge losses to almost all kinds of legumes in storage conditions (Prajapati *et al.*, 2023). In India, *Callosobruchus* spp. commonly known as pulse beetle. Numerous legumes are infested in India by seventeen species of pulse beetles representing eleven genera (Arora, 1977). Grain pulses are infected by the genus *Callosobruchus* both before and after harvest across the world. The two most common species of these genera in India are *C. chinensis* and *C. maculatus* (Dias and Yadav, 1988).

In the world, around 2400 species of plant have been identified as having possible protective qualities against a variety of pests (Grainge and Ahamed, 1988). Plant-derived products are less toxic to humans, reduce the development of resistance and may be more selective in action and alternate to chemicals for longterm storage of grains. Their primary benefit is that farmers and small-scale enterprises can produce them cheaply. According quickly and to several investigations, mixing stored grains with plant leaves, bark, seed, or oil extracts decreased the rate of oviposition, suppressed the emergence of adult pulse beetles and declined the amount of seed infestation (Parmar et al., 2018; Senthilraja and Patel, 2021). For the control of the pulse beetle, several spice powders have been claimed to have insecticide qualities, and among them, black pepper and clove have proved to be the most efficient (Mahdi, 2016). Hence, as ecofriendly substitutes for synthetic chemicals against pulse beetles, products derived from plants can be suggested in the food security program.

This pest infests mostly all pulses in the storehouses and grain shops and causes a loss in seed weight and viability, decreases germination rate and reduces the nutritional as well as the market value of the product. So, the utilization of plant-derived resources for the management of storage pests particularly in edible commodities is most efficient and eco-friendly method. Considering the seriousness of this pest and to develop economic and effective control measures, the present study was conducted.

Materials and Methods

The trial was performed throughout 2021-22 at the Laboratory of Entomology, Pulses Research

Station, SDAU, Sardarkrushinagar (Gujarat). For investigation, healthy, insect-pests free and genetically pure seeds of pigeonpea were attained from the Pulses Research Station, Sardarkrushinagar (Gujarat).

Test insect culture

An adequate quantity of *C. maculatus* stock culture was collected from the Department of Entomology, Pulses Research Station, SDAU, Sardarkrushinagar. The adults were allowed to hatch from eggs that were deposited on pulse seeds after being placed in plastic containers wrapped through muslin cloth. This standard culture was maintained at room temperature.

Sr. No.	Botanical powders	Botanical name	Family name	Dose (g/kg of seed)
1	Clove powder	Syzygium aromaticum L.	Myrtaceae	25
2	Black pepper powder	Piper nigrum L.	Piperaceae	25
3	Nutmeg powder	Myristica fragrans Hout.	Myristicaceae	25
4	Turmeric powder	Curcuma longa L.	Zingiberaceae	25
5	Red chilli powder	Capsicum annuum L.	Solanaceae	25
6	Cumin powder	Cuminum cyminum L.	Apiaceae	25
7	Green cardamom powder	Elettaria cardamomum	Zingiberaceae	25
8	Dill seed powder	Anethum graveolens L.	Umbelliferae	25
9	Neem leaf powder	Azadirachta indica	Meliaceae	25

Table 1: List of powders evaluated as grain protectants against C. maculatus

Preparation of powders

The required quantity of materials (Table 1) was obtained from a market near the SDAU, Sardarkrushinagar. Materials were crushed with an electric grinder into powder. After crushing the material powder was sieved frequently to get the uniform particle and each powder was stored in different plastic containers for more study.

Procedure

100 gm of pigeonpea seeds were taken into plastic containers for each repetition. The different plant powders were uniformly mixed with the seeds in plastic containers. Five (5) sets of the recently emerged male & female of *C. maculatus* were put into separate plastic jars which were protected through muslin cloth and tied through an elastic band. Three replicates of each treatment, including the control, were stored. The adults were allowed to stay in the jar freely till they died naturally at room temperature. The following measurements were made after a storage period of 60 days.

Egg laying

After the beetle's death, 100 seeds at random from each treatment were chosen to count the number of eggs deposited within each treatment.

Fecundity inhibition (%)

Fecundity inhibition (%) was calculated through the following equation given by Pascual-Villalobos and Robledo (1998).

Fecundity inhibition (%) =
$$\frac{1 - \text{No. of eggs in treatment}}{\text{No. of eggs in control}} \times 100$$

Adult emergence

Fifteen to twenty days onward specimen jars were observed daily to record the number of beetles emerged within each treatment.

Seed damage (Seed infestation)

These were measured by counting the number of seeds damaged out of 100 randomly chosen seeds within each treatment. They were used for finding the percent seed damage. The following formula was used (Adams and Schulten, 1978).

Seed damage (%) =
$$\frac{\text{No. of damaged seed}}{\text{Total no. of seed selected}} \times 1000$$

Weight loss (%)

After eliminating the dead pulse beetles from the container, the final weight was taken with electric balance separately for each treatment. The weight loss (%) was calculated with the below-described equation given by Dabi *et al.* (1979).

$$WL = \frac{I-F}{I} \times 100$$

Where, WL = Weight loss (%); I = Initial weight of seeds; F = Final weight of seeds.

Feeding Index: The formula described below was used to determine the feeding index:

$$FI = \frac{Wc - Wt}{Wc} \times 100$$

Where, FI = Feeding Index (%); Wc = Weight loss in control; Wt = Weight loss in treatment.

Seed germination (%)

By collecting twenty-five seeds from each container, the impact of powders on germination was measured. The seeds were put in a Petri dish on wet blotting papers. The blotting paper was then kept at 25 ± 10 °C temperature in room conditions. The ISTA technique was used to determine the germination percentage (ISTA, 1985).

Germination (%) =
$$\frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Statistical analysis

Every experimentation was carried out utilizing completely randomized design and it was repeated thrice. The data were transformed to percentage and absolute values using either an angular or square root transformation. Data were analyzed using GRAPES data analysis tool (Gopinath *et al.*, 2020). Significant variances among treatments were determined by DNMRT (Steel and Torrie, 1980).

Results and Discussion

Effect on oviposition

The data on the number of eggs laid by *C. maculatus* in stored pigeonpea treated with various powders are presented in Table 2. The outcomes exhibited that the treatment of different powders was found to be superior as compared to control and egg laying ranged between 0.00 to 45.00 in all treatments. Among all treatments, seeds treated with black pepper powder were free from eggs of pulse beetle (C. maculatus) at sixty days after storage. It was followed by clove powder (7.62 eggs) which was at par with neem leaf (9.30 eggs) followed by nutmeg (22.97 eggs), turmeric (28.30 eggs), dill seed (31.99 eggs) and cumin (37.66 eggs) which were significantly differed from each other. Cumin powder was at par with green cardamom (37.66 eggs) followed by red chilli (45.00 eggs), which was almost significantly less than eggs laid in untreated control (59.33 eggs). Among the different treatments, black pepper powder at 2.5 g per 100 g seed provided complete protection of pigeonpea seeds from C. maculatus. On the other hand, green cardamom and red chilli powder at 25 g/kg recorded maximum number of eggs.

The current results are in agreement with Pathania and Thakur (2013) who also stated that seeds treated with black pepper powder alone at 3 and 5 g/kg seeds provided complete protection (no oviposition) against pulse beetles after five months of storage. Equivalent results were also testified by Mahdi (2016), Ahmed et al. (2016) and Thakur and Pathania (2020) who found that seeds treated with black pepper powder and clove powder had the lowest oviposition by pulse beetles. Samuel et al. (2016) found that black pepper contains the alkaloid piperine and has larvicidal activity. Cloves are the highest source of phenolic substances, as well as gallic acid, eugenol and eugenol acetate (Cortes-Rojas et al., 2014). They also possess larvicidal, antimicrobial and antioxidant qualities. According to Swamy and Raja (2018), black pepper (P. nigrum) has an active ingredient called piperine, which is the main essential principal chemical in seeds that kills C. maculatus.

Fecundity inhibition (%)

The data on the percent fecundity inhibition of *C. maculatus* in stored pigeonpea treated by different powders are presented in Table 2. The outcomes showed that the significant difference among different powders and percent fecundity inhibition ranged from 21.34 to 92.25 percent in different treatments. Based on the fecundity inhibition of *C. maculatus*, pigeonpea seeds treated with black pepper powder were most promising against pulse beetle as they recorded maximum fecundity inhibition (92.25%). While minimum fecundity inhibition (21.34%) was recorded in pigeonpea seed treated with red chilli powder.

Tr.	Treatments	Number of aggs laid	Fecundity inhibition (%)	Number of adult
No.	Treatments	Number of eggs latu		emergence
T ₁	Clove powder	2.85 ^b	67.80	2.54 ^b
		(7.62)*	(85.68)**	(5.97)*
T_2	Black pepper powder	0.71^{a}	73.87	0.71^{a}
		(0.00)	(92.26)	(0.00)
T ₃	Nutmeg powder	4.84 ^c	50.12	4.30°
		(22.97)	(58.87)	(17.99)
T ₄	Turmeric powder	5.37 ^d	44.69	4.95 ^d
		(28.30)	(49.47)	(23.95)
т	Red chilli powder	6.74 ^g	27.45	6.26^{f}
15		(45.00)	(21.34)	(38.66)
т	Cumin powder	6.18 ^f	35.24	5.08 ^{de}
16		(37.66)	(33.30)	(25.33)
т	Green cardamom powder	6.28^{f}	33.73	5.34 ^e
17		(39.00)	(30.87)	(27.98)
T ₈	Dill seed powder	5.70 ^e	40.83	4.85 ^d
		(31.99)	(42.77)	(22.99)
T ₉	Neem leaf powder	3.13 ^b	65.89	2.73 ^b
		(9.30)	(83.30)	(6.98)
T ₁₀	Untreated control	7.73 ^h	4.05	6.89 ^g
		(59.33)	(0.00)	(46.99)
S.Em. ±		0.10	1.49	0.09
C. D. at 5%		0.28	2.87	0.26
C.V. %		3.34	3.80	3.50

Table 2: Effect of grain protectants on oviposition, fecundity inhibition and adult emergence of C. maculatus

*Figures within parentheses are real values and outside are $\sqrt{x+0.5}$ transformed values;

**Values outside of parenthesis are arcsine transformed values and values inside are real values;

Values followed by the same uppercase letter(s) on the same column do not significantly differ as per DNMRT ($p \le 0.05$).

These outcomes were also followed by Govindan *et al.* (2023) who found that black pepper seed powder @ 2% acts as the best oviposition deterrent with no egg laying of *C. maculatus* on stored blackgram. The current results corroborate those of Manju *et al.* (2019), who found that seeds of greengram treated with black pepper powder at 1% exhibited the maximum oviposition inhibition (71.6%) of *C. maculatus*.

Adult emergence

The data on adult emergence from C. maculatus infested pigeonpea seed after two months of storage period are presented in Table 2. The number of adults produced by each treatment against C. maculatus was significantly lower than the control treatment (46.99). The sequence of adult emergence at 25 g/kg in different treatments of powders were: black pepper (0.00) > clove (5.97) > neem leaf (6.98) > nutmeg(17.99) > dill seed (22.99) > turmeric (23.95) > cumin (25.33) > green cardamom (27.98) > red chilli (38.66). No adult emergence was recorded on black pepper treated seeds followed by clove powder which in turn was at par with neem leaf powder. Dill seed powder followed the series and was statistically at par with turmeric and cumin followed by green cardamom powder. Red chilli powder was significantly less

effective as compared to other plant powders. The outcomes of the current study are parallel with the results of Thakur and Pathania (2020), who also found that black pepper provided complete protection against pulse beetle at 3 g/kg and no adult emergence was detected in blackgram seed after 150 days of storage. Additionally, Ahmed *et al.* (2016) found that whenever chickpea seeds were mixed with clove powder, there was no adult emergence of *C. chinensis.* Similar results were also found by Govindan *et al.* (2023).

According to Devi and Devi (2013), wheat seed treated with black pepper and clove powder completely inhibited the F1 offspring of Sitophilus oryzae. In addition, Suthar and Bharpoda (2016) found that blackgram seed treated with neem leaf powder was efficient by decreasing the C. chinensis adult emergence. According to the results of Aslam et al. (2002), legume seeds treated with black pepper and clove powders observed a lower number of adult emergence of pulse beetle. Mahdi and Rahman (2008) reported similar findings and suggested that the active ingredients in both spices may be responsible for affecting the pulse beetle's (C. maculatus) physiological behavior viz., ovicidal activity, growth suppression and adult mortality.

Seed damage (%)

Tr. No.

The data on seed damage (%) due to bruchid infestation in pigeonpea after two months of storage is shown in Table 3. According to the outcomes, the percentage of seed damage in all the different treatments was significantly less than seed damage in untreated control. While evaluating the results obtained in different powder treatments, the average seed damage varied between 0.00 to 18.33 percent. The descending order of powders in which seed damage (%) was observed: red chilli powder (18.33) > green cardamom (16.33) > cumin powder (14.99) > turmeric powder (12.66) > dill seed powder (11.33) > nutmegpowder (8.98) > neem leaf powder (5.29) > clove powder (3.32) > black pepper powder (0.00). Black pepper powder was significantly most effective treatment. Dill seed powder was at par with turmeric powder. These were followed by cumin powder and was at par with green cardamom powder. While, significantly least effective treatment was red chilli powder as compared to rest of the powders tested.

These findings completely concur with those of Patil (2000) who found that black pepper powder completely protects the seed of green gram and no seed damage was observed after 2 months of storage. Venkatesham *et al.* (2014) also observed black pepper

Treatments

powder at 5g/kg seed was found effective after two months of storage and showed zero percent damage in chickpea seed. Similar results were found by Thakur and Pathania (2013), who also revealed that lowest percent seed damage was observed in black pepper powder treated seeds, also the oviposition deterrent activity against pulse beetles and higher mortality rate of adults. Similar results were also found by Mahdi and Rahman (2008), Mahdi (2016) and Keishing and Yadav (2023) who revealed that seeds treated with black pepper powder recorded a higher mortality rate in adults.

Weight loss (%)

Weight loss

Data on weight loss (%) due to *C. maculatus* damage in pigeonpea after two months of storage are presented in Table 3. The proportion of weight loss in all treatments was considerably lower than in untreated control. The chronological order of weight loss (%) in powders was: red chilli (17.90) > cumin (16.53) > green cardamom (15.56) > dill seed (13.20) > turmeric (13.10) > nutmeg (11.03) > neem leaf (6.11) > clove (0.60) > black pepper (0.00). Turmeric powder was statistically at par with dill seed powder followed by green cardamom powder which was at par with cumin. Red chilli powder was significantly less effective in comparison to the other powders tested.

Feeding index

Germination

Table 3: Effect of C. maculatus on grain protectant treated stored pigeonpea

Seed damage (%)

			(%)	(%)	(%)
T_1	Clove powder	10.50 ^b	4.43 ^a	80.35 ^b	72.29
		(3.32)*	$(0.6)^*$	(97.18)*	(90.75)*
T_2	Black pepper powder	4.05^{a}	4.05 ^a	85.95 ^a	71.54
		(0.00)	(0.00)	(100.00)	(89.97)
T ₃	Nutmeg powder	17.44 ^d	19.39 ^c	43.93 ^d	72.29
		(8.98)	(11.03)	(48.10)	(90.75)
T_4	Turmeric powder	20.85 ^e	21.22 ^d	38.24 ^e	69.91
		(12.66)	(13.10)	(38.35)	(88.20)
т	Red chilli powder	25.35 ^g	25.03 ^f	23.34 ^g	71.82
15		(18.33)	(17.90)	(15.70)	(90.27)
т	Cumin powder	$22.78^{\rm f}$	23.99 ^{ef}	28.01 ^f	70.19
16		(14.99)	(16.53)	(22.21)	(88.52)
т	Green cardamom powder	23.84 ^f	23.23 ^e	31.19 ^f	68.63
17		(16.33)	(15.56)	(26.78)	(86.72)
т	Dill seed powder	19.67 ^e	21.30 ^d	37.98 ^e	71.19
18		(11.33)	(13.20)	(37.88)	(89.60)
T9	Neem leaf powder	13.30 ^c	14.31 ^b	57.54 [°]	73.45
		(5.29)	(6.11)	(71.24)	(91.89)
т	Untreated control	28.88^{h}	27.45 ^g	4.05 ^h	70.01
1 ₁₀		(23.33)	(21.25)	(0.00)	(89.41)
S.Em. ±		0.44	0.37	1.13	2.92
C. D. at 5%		1.30	1.08	3.33	NS
	C.V. %	4.09	3.45	4.55	7.09

*Values outside of parenthesis are arcsine transformed values and values inside are real values; Values followed by the same uppercase letter(s) on the same column do not significantly differ as per DNMRT ($p \le 0.05$).

The result of the present experiment was supported by Thakur and Pathania (2013) who also recorded no loss in weight due to C. chinensis infestation in blackgram seeds treated through black pepper powder after five months of storage. Likewise, Govindan et al. (2023) found that black gram seed treated with black paper powder recorded zero percent weight loss by C. maculatus. Venkatesham et al. (2014), Ahmed et al. (2016), Pathania and Thakur (2020) and Keishing and Yadav (2023) also found the lowest weight loss (%) in seeds treated with black pepper and clove powder, which were the most effective treatments. The strong odors of cloves because of eugenol and in black pepper because of piperine potentially killed the beetles quicker (Javaid and Poswal, 1995; Devi and Devi, 2013).

Feeding index

The data on the feeding index of pigeonpea seeds at 60 days after treatment are shown in Table 3. The feeding index of different treatments fluctuated between 15.70 to 100.00 %. Significant difference was observed between the different treatments. Normally, treatment with a high feeding index is the best treatment. The ascending order of feeding index (%) was: red chilli powder (15.70) < cumin powder (22.21)< green cardamom powder (26.78) < dill seed powder (37.88) < turmeric powder (38.35) < nutmeg powder (48.10) < neem leaf powder (71.24) < clove powder (97.18) < black pepper powder (100.00). A significantly high feeding index was recorded in black pepper powder. Turmeric powder was statistically at par with dill seed powder followed by green cardamom powder which was at par with cumin powder. While, red chilli powder recorded the significantly lowest feeding index among different powders which was significantly higher than untreated control.

The current experiment's findings concur with those of Javed (2014), who obtained a higher feeding index from the treatment with the least percentage of weight loss. According to Senthilraja and Patel (2021), the treatment with the highest feeding index was the most effective. The feeding index fluctuated from 13.38 to 50.63 % for various dried leaf powder treatments. There was considerable variation among the treatments, and neem leaf powder had a considerably high feeding index. Therefore, from the above discussion, it is clear that the feeding index is negatively correlated with percent weight loss.

Effect on germination

The influence of several powders on the germination of pigeonpea seeds after two months of

period was investigated and outcomes are shown in Table 3. The results exhibited that the germination of pigeonpea seeds after treatment with various powders as protectants showed non-significant effect on germination. The proportion of germination among treatments fluctuated between 86.72 to 91.89 %. The chronological order of germination (%) of treated seed was: neem leaf (91.89) > clove (90.75) = nutmeg powder (90.75) > red chilli (90.27) > black pepper (89.97) > dill seed (89.60) > cumin (88.52) > turmeric (88.20) > green cardamom (86.72).

Suthar and Bharpoda (2016) recorded germination of blackgram before and after treatments and noticed that the variation in percentage of seed germinated uniformly before and after treatments, implying that there was no adverse impact of treatment on the germination of seeds after six months. Similar outcomes were also testified by Parmar *et al.* (2018), Manju *et al.* (2019) and Rathod *et al.* (2019) who detected that greengram seed treated with powders for three months had no detrimental impact on seed germination. Senthilraja and Patel (2021) also recorded that none of the botanical powders had an adverse impact on the germination of cowpea seed after two months of storage.

Conclusion

According to the research described above, it is evident that black pepper and clove powder which contained active components like piperine and eugenol, respectively acted as a repellent, a deterrent to oviposition and also caused adult mortality. Which directly affected the pulse beetle (*C. maculatus*) by reducing oviposition and adult emergence. These are becoming a potential alternative to chemicals for costeffective and ecofriendly pest management method against *C. maculatus* in stored pulses.

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