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Assessment of insecticidal potential of *Leucaena leucocephala* seed powder against *Callosobruchus maculatus* infesting stored black gram seeds

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Abstract
Callosobruchus maculatus is the ubiquitous and devastating pest of black gram seeds. The infestation starts from the fields and reaches up to the store houses. Synthetic pesticides are health hazardous and disturb the environment. Botanical pesticides are effective and promising alternative against stored grain pests. The present research was conducted with *Leucaena leucocephala* seed as powder. The results vary with different doses concentrations. During preliminary tests the number of adults emerged was found 51.4±3.12 adults at the dose of 5g/kg which was further followed with 66.2±3.2, 200.0±4.47, 211±4.30 at the dose of 10.0, 2.5, and 1.25 respectively. The adult mortality was 86% at the dose of 10g/kg. Similarly, the inhibition rate and egg reduction% were recorded 90.93% and 63.97% respectively at the dose rate of 10g/kg. In the ovicidal test, inhibition % were found 100 % with *Leucaena* seed powder against 100 egg bearing black gram seeds at 20 g/kg and no adult was emerged. With further lower doses the results was also promising comparatively untreated control. However *Leucaena* seeds were also effective at 10g/kg and show no adult emergence. In larvicidal study with treatment of seed powder of *Leucaena*, the complete adult inhibition was found with 1st, 2nd and 3rd larval instars at 20g/kg dose. With 4th instar larva the inhibition % were found more than 90% but not complete.

Keywords: *Leucaena*, powder, *Callosobruchus*, mortality, oviposition, inhibition

Introduction
Pulses are the essential and significant commodity of mankind. Various pests deteriorate these pulses both in fields and stores and affect their Quantity and Quality. Among pulses black gram plays a significant role in human diet. It is originated in India, even grown and harvested in other countries of Asia. India ranked and enlisted as highest black gram producer and user. From the cultivated area of about 4.6 million hectares, there is approximate production of 24.5 lakh tons of black gram yearly. In the year 2020-21 the average production were found533 Kg/hectare in 2020-21 (agricoop.nic.in). At commercial scale the pulses are stored either in the farm or at storehouses during post-harvest stage. In storing condition the pulses are pulses are more prone to attack by several enemies and pests. *Callosobruchus maculatus* (Coleoptera: Bruchidae) were observed as globally destructive and devastating pest of various pulse seeds.
Pulses grains are heavily prone to insect pest infestation. During stored condition the beetle produce generations enormously and can cause grain weight loss approximately 60%. In India losses after harvesting and in storing conditions can be as much as 20-50 %. This can also leads up to 90 % with the increasing time periods (Allotey and Oyewo, 2004; Radha and susheela 2014; Ekeh *et al*., 2015) [5, 28, 10]. Adult females laid eggs on the surface of seeds grains which follows the hatching into first instar. This enters inside the grains complete moulting and emerged as adult through constructing window hole in grain (Kalpna *et al*., 2022) [18]. Seeds with infestation become unfit for prior use. To protect the grains, these insect pests should be controlled at early (egg and larva) developmental stages. Control measures include spraying and fumigation various synthetic chemicals which are toxic and unfit for society. Even these chemicals are costly for the small holders and require well equipment and trained people (Ilesanmi and Gungula, 2010) [16]. The heavy and frequent use of chemical insecticides creates the issue of resistant pest, and even harmful constituents reaches in food stuffs. This harm health and environmrnt of consumers (Owolabi, *et al*., 2014) [26]. In view of expensiveness of chemicals the economically weaker society did not use them. At this stage plants can be better and promising alternative for the control of *C. maculatus* infesting black gram seeds (Campos *et al*., 2014; Melo *et al*., 2015) [8, 22]. Botanical products drew the scientists' attention and found a prominence as providing highly active insecticidal compounds, against several insect pests as well stored grain pests. It acts as safer approach for environment and mankind and also a better alternative for chemical pesticides (Mishra *et al*., 2012; Isman and Grieneisen, 2014; Kedia *et al*., 2015) [23, 17, 19]. During past few years, organic farming has becomethe most significant and attention seeking concern of society. In which synthetic chemicals are avoided in crop production and storage. The current research was aimed to assess the insecticidal efficacy of the *Leucaena leucocephala* plant seed powder against *C. maculatus*.

Materials and Methods

Collection and Breeding of Bruchid Pest: To raise the culture in laboratory, the mother culture of the test insect *C. maculatus* was procured from NBAIR, Bangluru, Karnatka, India (Accession no. NBAII- MP- Bru-01-C- maculatus). Rearing was carried out at temperature (27.8±2.5ºC) and relative humidity (70.3±1.03%) in biological oxygen demand incubator. Freshly emerged adults were used for study.

Experimental seed sterilization and plant powder preparations

The black gram seeds were sterilized at 55-60ºC for 5 hours to kill any hidden infestation. The Different plants were gathered from Career Point University campus as well its environs. Seeds were air-dried for two weeks, ground separately and filtered through a 70- mesh size sieve.

Experimental design

Screening of different plant powders against *C. maculatus* to protect black gram seeds were carried out by following the modified protocol as earlier described by Hossain *et al*., 2014.

Each plant powder was applied at 10, 5, 2.5, and 1.25 per 50 g black gram seeds in separate glass beakers (250 ml) with five pair *C. maculatus* adults. Black gram seeds without plant powder were put into a container and served as untreated control. The beakers were properly tightened with muslin cloth.

Calculations

$$\textbf{Adult Mortality (\%)} = \frac{\text{Dead insects}}{\text{Insects introduced}} \times 100 \text{ (Abbott, 1925)}$$

$$\textbf{Oviposition Reduction (\%)} = \frac{\text{Eggs laid in control- Eggs laid in treated container}}{\text{Eggs laid in control}} \times 100$$

$$\textbf{Inhibition Rate (\%)} = \frac{\text{Cn-Tn}}{\text{Cn}} \times 100$$ (Shukla *et al*. 2007).

Where, Cn = Number of insect on control treatment, Tn = Number of insect on treated treatment.

$$\textbf{Seed Damage (\%)} = \frac{\text{Initial weight -weight of undamaged seeds}}{\text{Initial weight}} \times 100$$ (Girish *et al*., 1975)

$$\textbf{Seed Weight loss \%} = \frac{\text{UNd - DNu}}{\text{U (Nd + Nu)}} \times 100$$ (Lal, 1988)

Where, U = Weight of undamaged seeds, D = Weight of damaged seeds,
Nu = Number of undamaged seeds, Nd = Number of damaged seeds.

Ovicidal and Larvicidal Tests

100 egg and larva containing seeds were placed in each petri-dish and applied the powder with the doses of 20.0, 10.0, 5.0, 2.5, 1.25 g/kg black gram seeds along with control. The petri-dishes were remained as such in BOD until adult emergence.

Completely randomized design with five replications was applied for experiment temperature (27.8±2.5ºC) and relative humidity (70.3±1.03%) in biological oxygen demand incubator. The inhibition% was calculated according to Shukla *et al*., 2007 [29].

Statistical Analysis: The obtained data were statistically analyzed using analysis of variance by adopting suitable transformation, wherever required (Gupta, 1986) [15].

Results

The potential of plant seed powder on adult oviposition, pest emergence, adult mortality, reduction and inhibition are presented (Table 1, Fig.1 & 3). The result presented in this study shows that the *Leucaena*seed powders were capable to cause death in seven days and cause 86 % and 74 % mortality at the respective dose rate of 10 and 5 g/kg seeds. In untreated black gram seeds no mortality was observed. This adult mortality shows consistence effect on oviposition, emergence, and inhibition. The lowest average eggs were observed 258.6±3.08 eggs with seed powder at the dose of 5 g/kg black gram seeds. While adults emerged were found 51.4±3.12 adults at the dose of 5g/kg which was further followed with 66.2±3.2, 200.0±4.47, 211±4.30 at the dose of 10.0, 2.5, and 1.25 g/kg respectively. After the bio-assayed with, the results were observed regarding inhibition rate %, oviposition reduction %. In this case the inhibition rate and oviposition reduction% were recorded 90.93% and 63.97% respectively at the dose rate of 10g/kg. The inhibition rate recorded was 92.95%, 72.60%, 71.10% at the

dose of 5.0, 2.5, 1.25 g/kg. The reduction of egg% was found 65.52, 60.00, and 59.02 with further with similar lower doses.During the observation of seed damage and weight loss % the effective dose was 10.0 and 5.0 of *Leucaena* seed powder. The seed damage % was recorded 18% and 25.6% at the dose of 10 g/kg and 5g/kg black gram seeds respectively. With these similar doses the seed weight loss % was noted 5.35% and 7.49%. *Leucaena* seed powder found less seed protective with further lower doses. The seeds damage and seed weight loss % were noted lower in comparison to untreated control.

Table 1: Effect of *Leuceana leucocephala* seed powder against oviposition, adult emergence, pest mortality%, inhibition rate%. Oviposition reduction %, seed damage and weight loss % with *C. maculatus* infesting Black gram seeds (n=5)

LLSP Dose (g/kg w/w seeds)	No. of eggs laid Mean±SE	No. of Adult emergence Mean±SE	Adult mortality %	Inhibition Rate %	Oviposition Reduction %	Seed Damage %	Seed weight loss%
10	270.2±4.31	66.2±3.2	86	90.93	63.97	18	5.35
5	258.6±3.08	51.4±3.12	74	92.95	65.52	25.6	7.49
2.5	300.0±4.47	118.6±3.28	68	83.75	60.00	26.4	7.15
1.25	306±4.00	164.4±4.34	64	77.47	59.02	27.6	9.14
Control	750	730	0	-	-	74	27.27

Fig 1: Black gram with *C. maculatus* and *Leuceana leucocephala* seed powder (10.0 g/kg seeds)

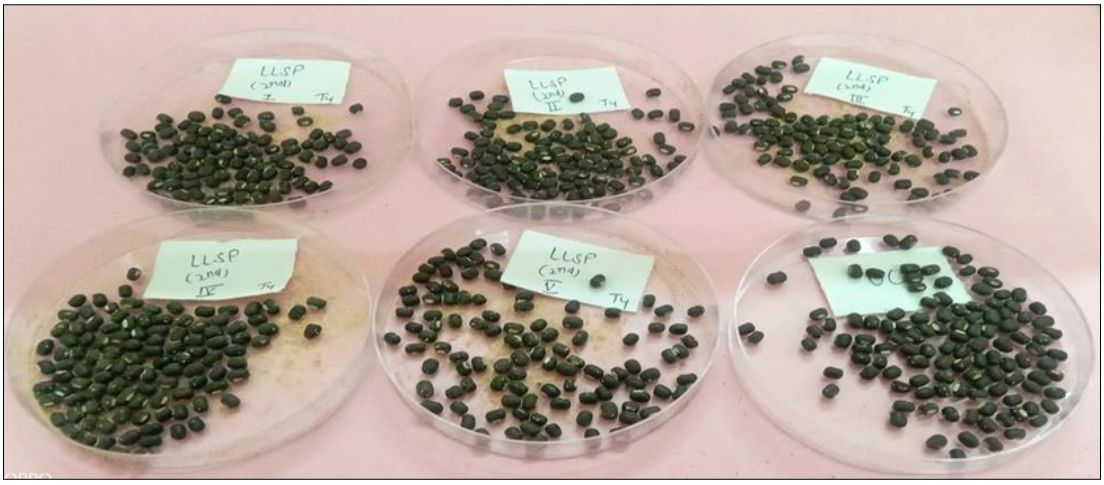

Fig 2: 2nd instar larva bearing black gram seeds with *Leuceana leucocephala* seed powder (10 g/kg seeds)

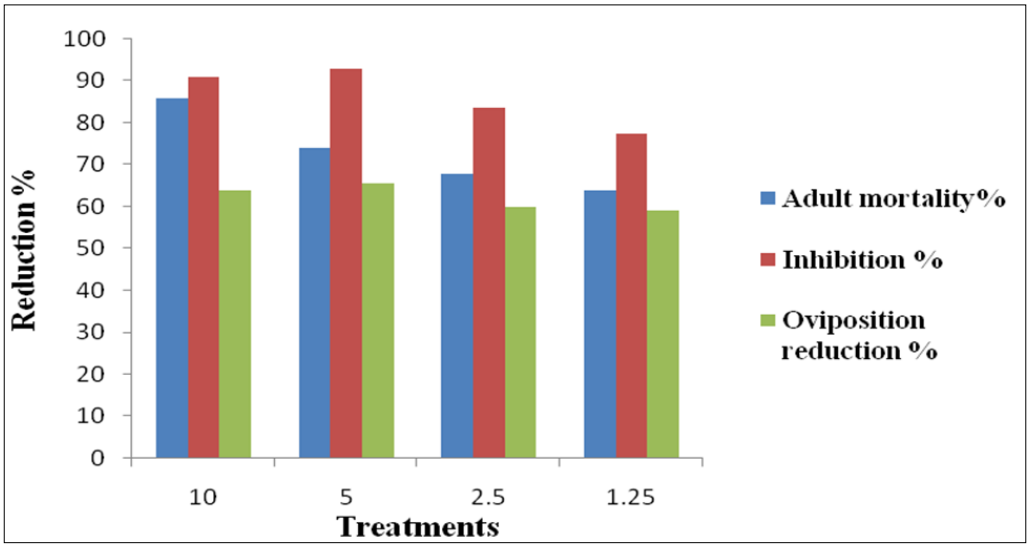

Fig 3: Effect of *Leuceana leucocephala* seed powder on adult mortality, inhibition and oviposition reduction

Ovicidal Study

Egg bearing black gram seeds was treated with *Leucaena* seed powder at different dose rate (20, 10, 5, 2.5, 1.25 g/kg). It was noted that the dose rate of 20 and 10g/kg were found highly effective as there was no adult emergence seen. And also 100% inhibition was found with these respective doses. The dose rate of 10g/kg seeds was also found 100 % effective for egg stage. The lowest inhibition was found with control in which egg bearing black gram seeds were not treated with powder (Table 2, Fig. 4)

Table 2: Number of adults of *Callosobruchus maculatus* emerged and inhibition % from 100 eggs (Ovicidal study) and larvae (larvicidal study) bearing black gram seeds after treatment with *Leucaena leucocephala* seed powder (n=5)

Ovicidal effect 100 egg bearing black gram seeds			Larvicidal effect 100 larva bearing black gram seeds							
			1st instar		2nd		3rd		4th	
Dose g/kg seeds	No. of adult emerged Mean±SE	Inhibition %	No. of adult emerged Mean±SE	Inhibition%	No. of adult emerged Mean±SE	Inhibition %	No. of adult emerged Mean±SE	Inhibition%	No. of adult emerged Mean±SE	Inhibition %
20	0	100	0	100	0	100	0	100	4±0.84	95.65
10	0	100	0	100	0	100	11±1	87.78	10.2±0.66	88.91
5	23.8±0.74	73.26	16.8±1.24	81.33	20.6±0.93	77.11	28.8±1.98	68	21±1.52	77.17
2.5	31.4±1.08	64.72	33.8±1.66	62.44	40.8±2.42	54.67	31.2±0.73	65.33	36.2±1.59	60.65
1.25	45.8±1.56	48.54	62.2±1.32	30.89	64.2±1.66	28.67	43.8±1.16	51.33	49.2±1.08	46.52
Control	89	-	90	-	90		90		92	-

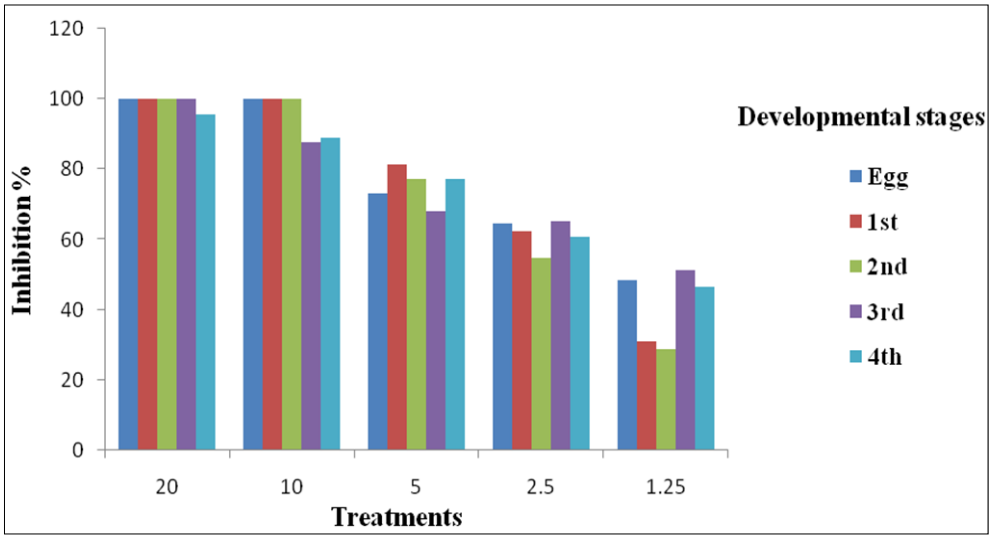

Fig 4: Effect of *Leuceana leucocephala* seed powder on developmental stages

Larvicidal Study

The larva bearing black gram seeds were also treated with different doses of *Leucaena* seed powder. The treatments against 1st, 2nd and 3rd Instars were found effective with 20 g/kg dose and found 100% inhibition and no adults were emerged. The 100% effectiveness of *Leucaena* seed powder against 1st and 2nd instars was also noted with 10 g/kg dose rate. With the treatments of *Leucaena* seed powder on 4th instar larva there was inhibition of 95.65 were found with doses of 20g/kg. There is approximately 88.91% inhibition with 10g/kg dose (Table 2, Fig. 2, 4).

Discussion

In present research, toxicity of *Leaucaena leaucocephala* seed powder evaluated against *C. maculatus*. The number of eggs laid (258.6±3.08) and adult emergence (51.4±3.12) was found lowest (258.6±3.08) with 5 g/kg seeds. While the results with 10 g/kg seeds were approximately similar with above concentrations. The inhibition rate % was noted more than 90% with 10 and 5 g/kg seeds. The oviposition reduction was found 60-65% with different doses. Fouad *et al*., 2020 [13]; Ileke *et al*., 2020 [15] also supported the effectiveness of botanical powder and found them as oviposition reduction agent, cause adult mortality and adult emergence of the pulse beetle. As the doses concentrations decreased the potential of seeds powder were also found to be decreased. It also causes adult mortality approximately 70-90 %. These findings were supported by Dele and Oyeteju, 2021 [9]; Sokker 2012 [30]. Shukla *et al*. (2007) [29] propounded the concept that powder can be lethal to *C. maculatus* because it blocks the spiracles of insects and leading to asphyxiation and death. The *Leaucaena leaucocephala* seed powder efficiently shows ovicidal and larvicidal property and cause 100% emergence inhibition. These results were supported by the Mohammadi Nori *et al*., 2018 [24]. They tested the different plant powders for their ovicidal and larvicidal effect on *C. maculatus* and *Zingiber officinalis* cause 80% egg mortality while 95.77% larval mortality. Lusiyana *et al*., 2018 [21] also provided the larvicidal activity of *Leucaena*. They conducted an experiment to test the efficacy of various extract of *Leaucaena leaucocephala*against *A. aegypti* early 4th instar larvae. It kills 50 and 90 % of treated larvae. The larvicidal property of the extract may be due to the presence of alkaloid, flavonoid and tannins compounds (Zayed and Samling, 2016) [31]. These botanical powders cause blockage to the respiratory holes of the eggs as well also toxic to immature and developing stages (Adedire & Lajide 2000). Various authors found the botanicals as seed protectant and reduce seed damage (Oparaeke and Dike, 2005;Baidoo *et al*., 2010; Brisibe *et al*., 2011; Ekah *et al*., 2013; Radha and Susheela, 2014; Petgrave *et al*., 2020) [25, 6, 7, 28, 27]. Figueiredo *et al*., 2020 [12] evaluated the efficacy some plants (*P. platycephala, L. leucocephala, and S. alata*) and also presented their ovicidal and larvicidal property. They mainly provide tannins and flavonoids, which can act alone or by synergism. Adama *et al*. (2012) [2] supported the findings by evaluating the ovicidal and larvicidal propery of *Leucaena* seeds. Ademola *et al*., 2005 [4] found that the most active fraction of the *Leucaena* seed are polyphenols, namely flavonoids and tannin.

Conclusion

The observations of current study concluded that *Leucaena* seed powder can be acts as potential bioorganic insecticides to control *C. maculatus*. The powder from seeds of *Leucaena* has insecticide action. This is because of that *Leucaena* seed powder, when tested against this pest, and then it causes, mortality, oviposition reduction, emergence inhibition etc. Moreover it also controls the pest at early developmental stages. The early larval stages are more voracious grain feeders, and make the grain empty. The *Leucaena* seed powder causes 100 % inhibition at developmental stages. Black gram protection can be provided with botanicals. So, this research provides a pathway and applicability of plant botanicals and their seeds part. This is less expensive, more effective and easily adapted technology by poor farmers and can be better alternatives for chemical insecticides. These results could be useful in eco-friendly formulations for insect control. This research also ensures for providing quality food grains. Although further investigations are need to interpret the active components, action strategies and interactions of this plant as well other plant natural products.

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