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Efficacy of Some Plants as Insecticide Against *Callosobruchus maculatus* Reared on Black Gram Seeds

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Abstract: Plant extracts/powders have potential to employ as promising and encouraged alternatives to synthetic insecticides in controlling stored grain pest. During present study, trials were carried out with five plants leaves powders [*Grewia optiva* leaf powder (GOLP), *Morus alba* leaf powder (MALP), *Murraya koenigii* leaf powder (MKLP), *Ficus carica* leaf powder (FCLP) and *Ricinus communis* leaf powder (RCLP)]. Highest insecticidal efficacy against oviposition and emergence of *Callosobruchus maculatus* were recorded after treatment with *Ricinus communis* leaf powder (RCLP). RCLP also offered maximum protection of black gram seeds applied at the dose of 20 g/kg seeds of black gram. Within seven days pest mortality percentile was maximum (86.67 %) as compared to other leaf powders. Whereas in untreated control, zero mortality was recorded. The lowest fecundity (186.6±3.91), pest emergence (95.4±2.89), seed damage (18.00%), and weight loss (5.28%) were noted with RCLP at the dose of 10.0 g/kg. In ovicidal treatment, RCLP inhibited the entire tested population emergence at 10 and 20 g/kg black gram seeds. The complete inhibition was also observed in 1st and 2nd instar larvae. Treatment of 3rd and 4th instar larvae with RCLP showed inhibition.

Keywords: Plant powder, *Callosobruchus maculatus*, Black gram, Infestation, Egg, Larva

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Introduction

Around the world, grains, pulses, cereals, and its significant products and by-products are chief and healthy food source; so, efficient protection of these principal food resources is needed. From the traditional time, different crops were grown as similar pattern and harvested. To increase the market value of grains, these were stockpiled in stored houses for prolonged time. With the time these valuable grains are exposed to several damage and losses done by the insect pest infestations. Internationally, stored grain pests provide the maximum damage (qualitative and quantitative) to commodities. The losses may be up to 40 % (Lorini and Filho, 2004; Fields, 2006). Around the world, *Callosobruchus maculatus* is a severe pest of pulses/grains especially in stores. Globally, Black gram seeds are immensely damaged by *Callosobruchus maculatus* (F.) as well

Callosobruchus chinensis (Hafez *et al*., 2014). *Callosobruchus maculatus* is vulnerable insect pest and also carried to storehouses with black gram at the time of harvesting. The heavy infested as well damaged grains become hollow and also lead to maximum loss of seed weight. Fully grown adult bruchid emerged out by making an emergence window after completion of larval-pupal growth stage (Kalpna *et al*., 2022). After few months, it provided inclusive damage to stockpiled grains. Approximately 30-40% damage and losses can be caused by bruchids (*Callosobruchus* spp.) within 6 months period and it can reach up to complete (100%) loss if remained unattended (Gbaye *et al*., 2011). To keep various stored pulses away from damage and spoilage, effective management strategies are required. Currently, most of peoples at large and small scale levels use harmful chemical insecticides. These insecticides with harmful chemicals adversely affect our surroundings and also hazardous to consumers (human as well as animals) at different aspects (Salem *et al*., 2007). At large scale, unsystematic use of various synthetic insecticides has also developed the resistance ability in these insects against such chemicals. Adverse consequences of the synthetic insecticides require assessment of botanical and other environment friendly materials and can be effectively used to control these ubiquitous pests without affecting and threatening to the mankind and flagging stored grain quality (Salem *et al*., 2007; Mahdi and Rahman, 2008, Alengebawy *et al*., 2021). There are various plants as well their parts and compounds, being organic, environment friendly and natural, which may create a pathway to initiate such research. Considering these aspects, Musa and Uddin (2016), Obembe and Ojo (2018) and Ileke *et al*. (2022) recently studied and identified powders/extracts of some plants having insecticidal properties against stored grain pests especially *Callosobruchus maculatus.* Such botanicals exhibit different feeding and oviposition deterrent properties which suppress the pest by repelling and expelling away. Presently many researchers have designed plant based pest management strategies (Uyi and Adetimehin, 2018; Ileke, 2021; Ashamo *et al*., 2021). Recently powder form of some plants parts were studied against *Callosobruchus maculatus.* These are the well authorized example of such botanical compound especially in powder form. With the increasing treatment doses it was observed that the deterrent and repellent activity also increased against insects (Harshani and Karunaratne, 2021; Kavitha and Poornima, 2021). Plant based products are inexpensive and are readily accessed by growers and farmers and also in small-scale business. Recently various authors used plant parts and products in powder/ oil form (Sanon *et al*., 2018; Manju *et al*., 2019; Soe *et al*., 2020, Fouad *et al*., 2020). Due to global population explosion, there is increase in universal requirement for healthy grain production. This produces colossal pressure for small holders to big farmers to enormous crop yields and also enhancement and exorbitant use of chemical pesticides, even without concerns for its long-term effects. Therefore, to avoid the side-effects of synthetic insecticides and their use, without imperil agricultural results outcomes, research for most effective options began in the last few decades and is also presently ongoing. Various plants produce significant compounds to conquer the challenges foisted by nature and are found as the main source of answers to this issue.

In this study, we observed the efficacy of *Grewia optiva* leaf powder (GOLP), *Morus alba* leaf powder (MALP), *Murraya koenigii* leaf powder (MKLP), *Ficus carica* leaf powder (FCLP) and *Ricinus communis* leaf powder (RCLP) against *C. maculatus*. Therefore, these were experimentally tested against different parameters of *C. maculatus* and black gram seeds such as fecundity, emergence, mortality, seed damage and weight loss etc.

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±2.5 C) and relative humidity (70.3±1.03%) in biological oxygen demand incubator. Freshly emerged adults were used for study.

Seed sterilization and plant powder preparations:

The black gram seeds were sterilized at 55-60 C for 5 h to kill any hidden infestation. Different plants (*Grewia optiva, Morus alba*, *Murraya koenigii*, *Ficus carica* and *Ricinus communis*) were gathered from Career Point University campus and its environs (Table 1). Leaves 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). GOLP, MALP, MKLP, FCLP and RCLP were tested against *C. maculatus*. During primary screening each plant powder was applied at 20 g per 50 g black gram seeds in separate glass beakers (250 ml) with five pairs of *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. Completely randomized design with three replications was applied for experiment temperature (27.8±2.5 C) and relative humidity (70.3±1.03%) in biological oxygen demand incubator. From the primary screening, the most effective powder were taken and further tested for secondary screening at lower doses (10, 5, 2.5, 1.25 g/kg black gram seeds) with 5 replications and control.

Calculations:

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

$$\text{Seed Damage (\%)} = \frac{\text{Initial wt} - \text{wt of undamaged seeds}}{\text{Initial wt}} \times 100$$

(Girish *et al.*, 1975)

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

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

Ovicidal and Larvicidal activity:

100 egg and larva containing seeds were placed in each petri-dish and applied the most effective powder with the doses of 20, 10, 5, 2.5 and 1.25 g/kg black gram seeds along with control. The petri-dishes were remained as such in BOD until adult emergence.

Calculations:

$$\text{Inihibition (\%)} = \frac{\text{(Control -Treatment mean)}}{\text{Control}} \times 100$$

(Shukla *et al*., 2007)

Statistical Analysis:

The obtained data were statistically analyzed by adopting suitable transformation.

Results

Primary screening:

Fecundity, pest emergence, grain damage and weight loss per cent were differed with all plant powders even at the same dose rate (20 g/kg black gram seeds). Maximum fecundity and pest emerged were noted in untreated control (730 and 700, respectively) while the minimum (169± 2.08 and 27.33±4.05) was in *Ricinus communis* leaf powder (RCLP). Again, the highest black gram seed damage (72%) and weight loss (26.35 %) were noticed in the control. RCLP (20 g/kg seeds) caused minimum seed damage and weight loss (12.0 and 3.98%) within 45 days. The highest adult pest mortality percentile was noted with RCLP while there was no adult found dead with untreated control (Table 2).

Secondary screening:

The RCLP was found most effective among all the treated plants. The further treatments were bio-assayed at lower doses.

Table1: Details of various plants used for evaluating toxicity against *Callosobruchus maculatus*

Botanicals	English name	Family	Parts used	
Grewia optiva	Bhimal	Tiliaceae	Leaves	GOLP
Morus alba	Mulberry	Moraceae	Leaves	MALP
Murraya koenigii	Curry Leaves	Rutaceae	Leaves	MKLP
Ficus carica	Common fig	Moraceae	Leaves	FCLP
Ricinus communis	Castor bean	Euphorbiaceae	Leaves	RCLP

Table 2: Effects of plant powders on oviposition, pest emergence, mortality, seed damage, and weight loss caused by *Callosobruchus maculatus* on black gram seeds (n=3)

Plants powder (20 g/kg seeds)	No. eggs laid Mean ± SE	No. of adult emerged Mean ± SE	Adult mortality (%)	Seed Damage (%)	Seed weight loss (%)
GOLP	651.33±2.03	641.67±4.41	46.67	64.93	22.01
MALP	662.33±4.33	635.00±4.04	43.33	63.33	22.01
MKLP	403.33±4.41	187.33±2.67	73.33	19.33	6.46
FCLP	561.00±5.51	344.33±3.48	66.67	42	12.26
RCLP	169±2.08	27.33±4.05	86.67	12	3.98
Control	730	700	0	72	26.35

Effect of RCLP on fecundity, pest emergence, grain damage and loss:

Fecundity: With different treatments fecundity of female *C. maculatus* was highly affected at the dose rate of 10.0 g/kg seed and the lowest eggs (186.6±3.91) were observed. Again, the maximum eggs were deposited in the control without RCLP (750.0), and followed at the dose of 1.25 g/kg seeds treated with RCLP (297.6±5.13) (Table 3; Fig. 1).

Pest emergence: During the treatment of black gram seeds with RCLP at the dose rate of 5.0, 2.5 and 1.25 g/kg grains the pest emergence were noted 169.2± 2.48, 184.0±3.67 and 189.0±3.32, respectively. The dose rate of 10 g/kg were found effective and observed lowest (95.4±2.89) emergence. The number of adults was recorded maximum in control (730.0) (Table 3; Fig. 1).

Seed damage and weight loss: Among all the treatment of RCLP with different doses the variations were observed in seed damage and loss caused by *C. maculatus*. The highest damage percentage (74.00) was noted with control and minimum (18%) with 10.0 g/kg dose. Similarly, the weight loss percentage of black gram seeds was found maximum with control. Lowest seed losses were found with RCLP applied at 10.0 and 5.0 g/kg seed (Table 3).

Ovicidal Effect:

RCLP were found 100% effective with dose rate of 10 and 20.0 g/kg seed and act as protectant of 100 black gram seeds containing eggs. No adult *C. maculatus* was emerged with this treatment within 30 days. RCLP was also effective at other lower doses (Table 4; Fig. 2).

Larvicidal effect:

During larvicidal study of 1st and 2nd instar larva the complete inhibition was occurred with 20g/kg seeds while the lowest at 1.25 g/kg seeds. While during the study with 3rd instar larva, the inhibition (92.44%) was found with similar dose.

Table 3: Effects of *Ricinus communis* leaf powder on oviposition, adult emergence, mortality, seed damage, and weight loss caused by *Callosobruchus maculatus* on black gram seeds (n=5)

Dose (g/kg seeds) RCLP	No. eggs laid Mean ± SE	No. of adult emerged Mean ± SE	Adult mortality (%)	Seed Damage (%)	Seed weight loss (%)
10	186.6±3.91	95.4±2.89	86.0	18.00	5.28
5	254.0±4.30	169.2±2.48	74.0	21.99	7.49
2.5	286.0±4.0	184.0±3.67	68.0	24.96	12.42
1.25	297.6±5.13	189.0±3.32	62.0	25.36	12.65
Control	750.0	730.0	0	74	27.27

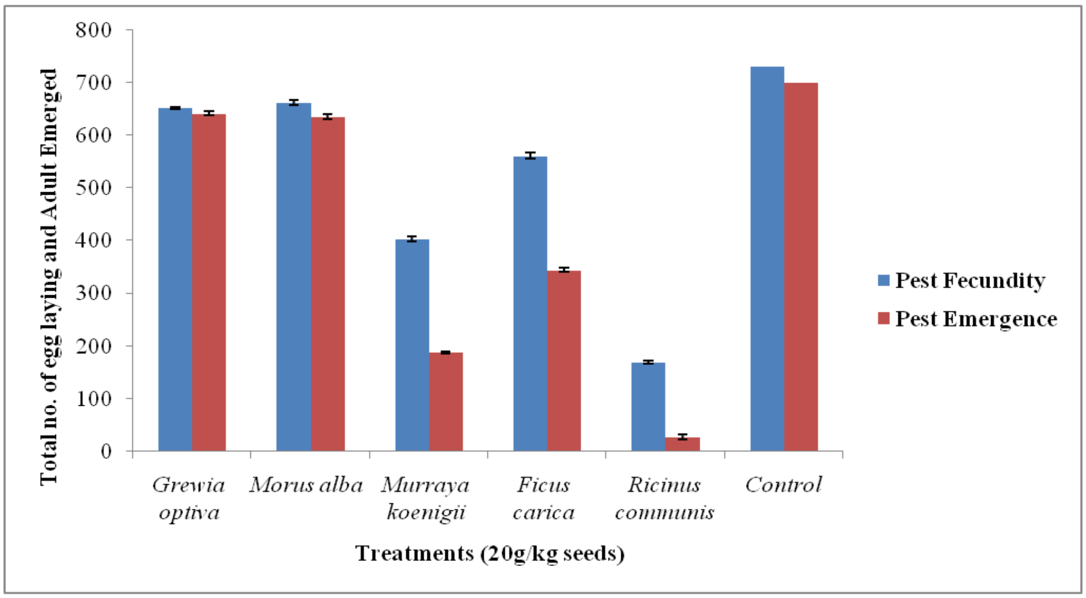

Fig. 1: Effect of different plant powder on fecundity and adult emergence of *Callosobruchus maculatus* infesting black gram seeds.

The results were comparable similar with the results of 1st instar larva (92.22%) at 10 g/kg seeds. While less inhibition was occurred with 4th instar in comparison to other treatments (Table 4; Fig. 2)

Discussion

In the present study RCLP showed toxicity in *C. maculatus* adult as well as in developing stages. The minimum egg laying occurred after treatment with 20 g/kg w/w and few eggs (169±2.08 eggs) were deposited. Treatment with lower dose of RCLP also showed effectivity on oviposition in comparisons to other treated plant powders. These results derive support from the findings of Miah *et al.* (2013), who have reported decreased oviposition of female *C. maculatus* after the treatment with RCLP at the rate of 5% w/w. the present findings are also supported by observations of Shitu *et al.* (2020) who have used *Ricinus communis* extracts. The RCLP also had promising effect on number of adult emerged (27.33±4.05) and adult mortality (86.67) at 20 g/kg seeds, while there was 0% mortality in control. The results of powder were also found effective at the treatment of lower doses of 10

Table 4: Number of adults of *Callosobruchus maculatus* emerged and inhibition % from 100 eggs and larvae bearing black gram seeds after treatment with *Ricinus communis* leaf powder (RCLP) (n=5)

Ovicidal effect **100 egg bearing black gram seeds**			**Larvicidal effect** **100 larva bearing black gram seeds**							
Dose g/kg seeds (RCLP)	**No. of adult emerged**	**Inhibition %**	**1st instar**		**2nd instar**		**3rd instar**		**4th instar**	
			No. of adult emerged	**Inhibition %**	**No. of adult emerged**	**Inhibition %**	**No. of adult emerged**	**Inhibition %**	**No. of adult emerged**	**Inhibition %**
20	0	100	0	100	0	100	6.8±0.58	92.44	22.2±1.24	75.87
10	0	100	8.8±0.37	90.22	17.4±0.75	80.67	32.0±1.41	64.44	49.4±2.36	46.30
5	31.6±1.50	64.49	18.0±1.22	80.00	47.8±1.69	46.89	45.0±1.41	50.00	60.08±2.48	33.91
2.5	52.8±1.66	40.67	53.6±1.33	40.44	60.8±2.42	32.44	75.2±2.47	16.44	73 ± 1.41	20.65
1.25	76.2±1.39	14.38	82.2±1.56	8.67	83±1.58	7.78	82.2±0.86	7.56	83.6±1.81	9.13
Control	89	-	90	-	90	-	90	-	90	-

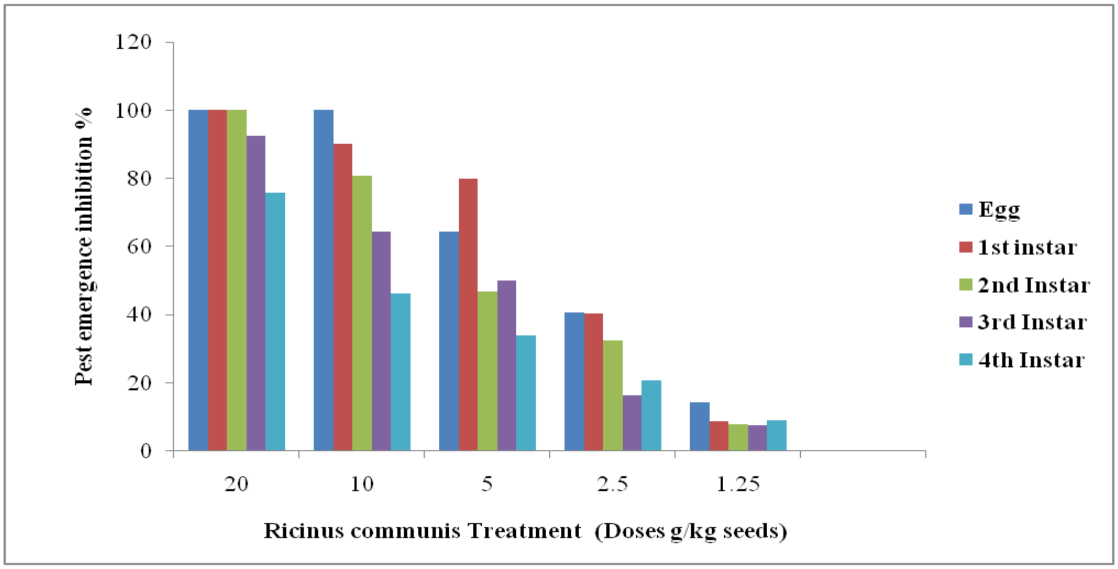

Fig. 2: Emergence inhibition % with treatment of *Ricinus communis* leaf powder on black gram seeds having eggs and larva of *C. maculatus.*

g/kg and 5 g/kg seeds. Latif *et al*. (2012) also reported that plant powder can act as adult pest emergence inhibitor.

Hossain *et al*. (2014) also noticed similar findings as observed in the present study when *C. chinensis* infesting chickpea seeds were treated with castor (20 g/kg w/w). The aqueous extract of leaves of *Ricinus communis* caused lowest percentage of adult emergence (30.90%) (Mahmoud *et al*., 2020). The RCLP treatment also showed effect on seed damage and weight loss in black gram seeds infested by *C. maculatus*. The results of present study are strengthened by findings of Rouf *et al*. (1996) who have reported that some botanicals (Neem, Nishinda and Biskatali) act as seed protectant. Babarinde *et al*. (2011) recorded 100% larval mortality with RCLP extracts at the dose of 0.3 ml/5 g seed against *Tribolium* sp. Upasani *et al.* (2003) found *R. communis* leaf extracts as better oviposition deterrent for *C. chinensis*, and number of adults emergence almost nil at 6.5 mg ml^{1} as compared to untreated and solvent controls. Similarly, Lal and Deepshika (2012) and Anam Sarwar *et al*. (2019) reported the use of castor and neem as grain protectant and also as complete inhibitor of adult emergence.

During ovicidal and larvicidal study the RCLP showed 100% inhibition in adult emergence when treated with 10 and 20 g/kg seeds while results with other lower doses treatments also had efficient and promising outcomes. Similarly castor oils showed 100% reduction in adult emergence in *C. maculatus* infesting moong bean. These powders/oils may act as toxic to eggs and larvae and entirely inhibit the pest emergence (Anam Sarwar *et al*., 2019). Bamaiyai *et al.* (2007) reported that plants exhibit ovicidal activities which abolish the developing pulse beetle. *Ricinus communis* leaves contain excellent insecticidal property. They have flavonoids (flavonol type) that have astonishing ovicidal as well deterrent activities for *Callosobruchus chinensis* (Upasani *et al*., 2003).The performance of botanicals as suppressant against pulse beetle observed in this study was also earlier reported by Shaaya *et al*. (1997). The seed damage rate for stored grains was also reduced while treated with botanicals.

Among other tested botanicals, MKLP also showed significant results for egg laying, adult emergence, adult mortality and pulse beetle infestation at 20 g/kg seeds. The other plants used in the study did not show efficient results for inhibition of pest emergence, grain damage and loss at the dose of 20 g/kg seeds. In this study diverse toxic efficiency of plant powders did not consistently showed the inhibition and lethal activities. Various botanicals such as castor, neem leaf, seed powder and other agriculture waste powder (rice and wheat husk) etc. provide convincing results against *Callosobruchus* sp. (Ileke *et al*., 2013; Edwin and Jacob, 2017; Ashamo *et al*., 2021; Gbate *et al*., 2021).

RCLP when directly applied to black gram seeds then it purely attached on seed surface and evenly distributed. This severely affects the *C. maculatus* (egg, larva and adults). These results are supported by Lale and Alaga (2001). *R. communis* extracts were applied to larval and adult stages. High mortality was observed with larval stages because exoskeleton of adult insects was heavily sclerotized. So, the permeability of toxic materials in cuticle were high in larva as compared to adults. The plant powders have combination of various active insecticidal constituents, generally monoterpenoides (Regnault-Roger *et al*., 2002). These active constituents in botanicals showed different mode of action against pests and especially they affect nervous system. It also disturbs the GABAergic as well aminergic system and also obstruct the acetylcholinesterase activity (Tong and Coats, 2012; Abdelgaleil *et al*., 2016).

Conclusion

In this study, black gram seeds infested by *Callosobruchus maculatus* were exposed to various plant leaf powders. These findings provide great attention in which we found that metamorphosis of 1st and 2nd instar larva was completely inhibited by RCLP. RCLP showed effectiveness against all studied parameters including eggs laid, pest emergence, and mortality. Other plant powders could be explored in future to check their insecticidal potential. Highly effective plant powders can be recommended as combinations. Moreover, this is environment friendly, cost effective, readily available and harmless approach to control *C. maculatus* infesting black gram at farmer level. Active compounds of RCLP could also be isolated and such formulations should be used against pests. This study enhances the use of plants as insecticides and also for stored grain pest management strategy.

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