

Effectiveness of botanicals against *Callosobruchus maculatus* (F.) in stored cowpea seeds under laboratory conditions

S V Parmar^{1*}, M L Patel², I B Kapadia³, J H Chaudhary⁴

^{1*} M.Sc. (Agri.), Junagadh Agricultural University, Junagadh, Gujarat, India

² Krishi Vigyan Kendra, Junagadh Agricultural University, Targhadia, Gujarat, India

³ Wheat Research Station, Junagadh Agricultural University, Junagadh, Gujarat, India

⁴ Seed Spices Research Station, Sardarkrushinagar Dantiwada Agricultural University, Jagudan, Gujarat, India

Abstract

The study investigated the comparative efficacy of some botanicals viz., Neem seed kernel powder, Mint leaf powder, Custard apple seed powder, Neem oil, Castor oil, Karanj oil, Azadirachtin 10000 ppm are used in the suppression of *Callosobruchus maculatus* (F.) damage in stored cowpea seeds. The botanicals were applied at the rate of 5.0 g/kg seed in case of powders and 5.0 ml/kg seed in case of oils. The Azadirachtin 10000 ppm was applied at the rate of 1.5 ml/kg seed. The experiment was laid out in completely randomized design (CRD) of nine treatment replicated four times. All treatments recorded higher significant mortality than the experimental control. Out of the all botanicals investigated azadirachtin was most effective.

Keywords: Botanicals, *Callosobruchus maculatus* (F.) and Laboratory condition

Introduction

Cowpea belongs to the Fabaceae family and the Faboideae subfamily. It is native to Central Africa, but over time, it has adapted to the semi-arid and sub-humid tropical regions of Asia, including India. Nutritionally, cowpea is a powerhouse. It is rich in protein, with approximately 4.2 grams of protein per 100 grams of edible portion, making it an excellent source of plant-based protein, especially in vegetarian diets. Additionally, cowpea contains calcium (110 mg/100 g) and iron (4.7 mg/100 g), both of which are vital for bone health and preventing anemia. Cowpea is also high in carbohydrates, providing 60.3% of the total weight, making it a good source of energy. Due to its high protein content and other essential nutrients, cowpea is often referred to as “vegetable meat.” (Singh, 1983) [12]. Being leguminous, it also enriches soil fertility by fixing atmospheric nitrogen into the soil. The infestation of stored grain pests has made it harder to store cowpea seeds in recent years, resulting in significant losses throughout the storage process. *C. maculatus* is a primary grain beetle (Cherry *et al.*, 2007) [2] commonly known as “dhora and ghun” which is widely distribute throughout the world and causes considerable damage in stored cowpea. According to the Indian Minimum Seed Certification Standard (IMSCS), seed becomes ineligible for continued use in sowing if seed damage from storage pests is detected at a rate more than 0.5%. Therefore, seed that has been harmed by insect pests while being stored cannot be accepted at all. Consequently, in order to reduce insect pests during storage and extend the shelf life of seeds, pre-storage management techniques are now essential. Combining botanicals is a traditional Indian

practice that is used in both rural and urban regions. It is a simple, affordable and safe way to preserve grains like cowpea seeds that are specifically kept for domestic consumption. Furthermore, the botanical ingredients present a chance to create biological insecticides that are safe and effective enough to be included into integrated pest control plans. Because native plant materials decompose quickly and provide less of a threat to mammals, they have become a valuable tool in contemporary pest management techniques. An attempt will be made to assess the effectiveness of botanicals against cowpea seed infestations by *C. maculatus* during the current study course.

Materials and Methods

Eight different botanicals were (Table 1) evaluated for their seed protectant efficacy against *C. maculatus* based on periodical mortality and population growth. The laboratory experiment was conducted in Completely Randomized Design (CRD) with 4 repetitions on GC 4 variety of cowpea. The different oils and azadirachtin (10000 ppm) commercial formulation was purchased from the local market of Junagadh, while powder of botanicals used in experiment were prepared in laboratory from shade dried parts of respective plants. Each botanical materials was mixed or smeared to 1.0 kg previously sterilized cowpea seeds. An untreated and sterilized bulk of 1.0 kg cowpea seeds were kept as a control treatment. All the nine bulks (each of 1.0 kg) of cowpea seeds were stored in airtight plastic jar at room temperature and utilized for further experimentation.

Table 1: Details of botanicals evaluated for their seed protected efficacy against *C. maculatus* infesting cowpea seeds

Treatment No.	Treatments details	Dose
T ₁	Neem seed kernel powder	5.0 g/kg seed
T ₂	Neem leaf powder	5.0 g/kg seed
T ₃	Mint leaf powder	5.0 g/kg seed
T ₄	Custard apple seed powder	5.0 g/kg seed

T ₅	Neem oil	5.0 ml/kg seed
T ₆	Castor oil	5.0 ml/kg seed
T ₇	Karanj oil	5.0 ml/kg seed
T ₈	Azadirachtin 10000 ppm	1.5 ml/kg seed
T ₉	Control–Untreated seeds	-

Adult Mortality

To evaluate the efficacy of botanicals against *C. maculatus* based on periodical mortality of adults, a series of experiments was carried out during 2024-25 in laboratory condition. Each experiment was carried out at one month interval. For each treatment, 150 g sample of treated cowpea seeds was drawn from each repetition. The samples were filled in plastic bottle individually. Ten pairs (five to ten days old) adults of *C. maculatus* obtained from laboratory culture were released into each of the plastic bottle and each plastic bottle was covered with a piece of two-fold muslin cloth, which was held in position with rubber band. The observations on number of total adults was recorded after 7 days of adult release and per cent adult mortality was worked out. Insects showing movement of legs or antennae were considered as alive. This experiment was conducted for a period of nine months. The periodical data on per cent mortality was corrected by using Abbott's formula (Abbott, 1925) [1] mentioned below.

$$P = \frac{T - C}{100 - C} \times 100$$

Where,

P = Corrected per cent mortality

T = Observed per cent mortality in treatment

C = Observed per cent mortality in control

Population Growth

To evaluate the efficacy of botanicals against *C. maculatus* based on population growth, an experiment was carried out using same bulks of cowpea seed under different treatments. For each treatment, 150 g sample of treated cowpea seeds were drawn from each repetition. The samples were filled in plastic bottle individually. Ten pairs of *C. maculatus* (5 to 10 days old) were released in each bottle for egg laying and each bottle was covered with two-fold muslin cloth kept in position by means of rubber band to prevent the adults from escaping. The adults introduced for oviposition was removed from each bottle after 7 days of oviposition. The observations on population growth (number of live and dead adults) in each repetition were recorded after three, six and nine months of initial oviposition by 10 pairs of adults. Per cent mortality was worked out based on number of dead adults out of total adults developed after three, six and nine months of storage periods.

Results and Discussion

Adult Mortality

Among botanicals tested, azadirachtin 10000 ppm was found to be the best treatment against *C. maculatus*. These findings were supported by the records of Debashri and Tamal (2012) [3] who stated that azadirachtin could protect legumes from *C. chinensis* and *C. maculatus* infestation up

to 12 months of storage. It might be primarily due to the presence of active compounds (limonoids) at the highest concentration that was responsible for its insecticidal actions. Secondly, being an oil-based emulsified concentrate formulation, it acts as a physical poison. This oil could enter the trachea of insects causing anoxia and ultimately led to mortality (Don Pedro, 1989) [4]. This was clear when dead insects were observed to be coated with oils. Among the different neem formulations, neem seed kernel extract powder was found most effective in reducing the pulse beetle infestation (Rani and Mohan, 2007) [11]. In addition to this, the appreciable adult mortality percent was noticed in neem oil and neem seed kernel extract treated seeds. These results were in partial congruence with Wahedi *et al.* (2004) [14] who unveiled that neem oil and neem seed powder recorded the highest mortality of *C. maculatus* in cowpea. Neem oil has been shown to be highly effective against *C. maculatus* in stored grains. Raghvani and Kapadia (2003) [9] reported complete protection of grains, with maximum adult mortality and up to six months of infestation control,

Population Growth

The results after three, six and nine months of storage, all botanical seed protectants significantly outperformed the untreated control. This efficacy is likely due to the bioactive compounds in each botanical and their varied modes of action. Neem products contain azadirachtin (a potent tetranortriterpenoid), custard apple seeds have acetogenins, castor oil contains ricin and ricinine, karanj oil includes karanjin (a furanflavonoid) and mint provides menthol and menthyl acetate. Among these, azadirachtin was the most effective, consistently suppressing *C. maculatus* populations. Its superior performance was due to its multiple actions as an insect growth regulator, antifeedant, repellent, sterilant and oviposition inhibitor (Opende, 1986) [7]. Ramazeame *et al.* (2014) [10] reported that neem kernel powder applied at 5 g/kg seed can be effectively recommended for controlling pulse beetles during storage. Similarly, Parmar and Patel (2015) [8] observed significantly lower adult emergence in neem-treated seeds (3.22 adults), followed by castor (4.70) and groundnut oils (5.17). Kumar *et al.* (2017) [6] also found neem oil @ 2.5 ml/kg seed to be the most effective treatment against pulse beetle infestation. Furthermore, Hasan *et al.* (2021) [5] reported that among various botanicals tested, neem oil was the most effective in inducing adult mortality in pulse beetles. Singh *et al.* (2017) [13] stated that minimum seed damage was noticed in nimbecidine treated seeds which was followed by neem leaf powder, karanj oil, custard apple seed powder and castor oil and hence proved to be effective against *callsobruchus chinensis* in chickpea.

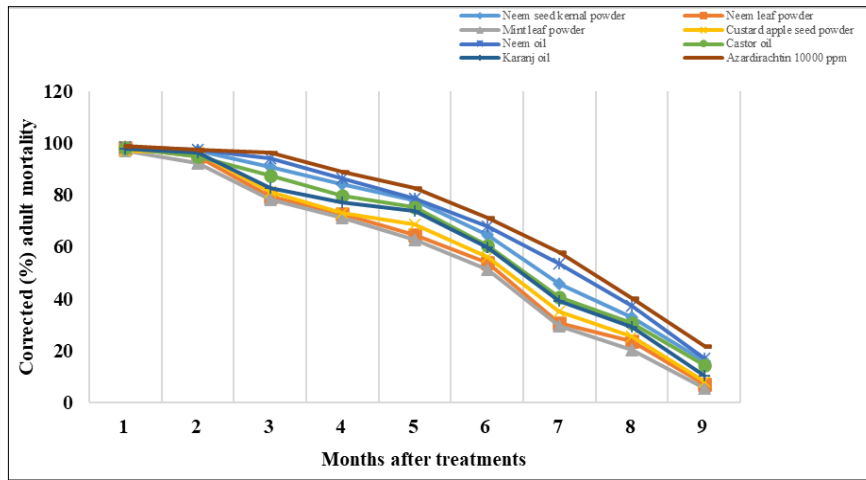


Fig 1: Corrected adult mortality of *C. maculatus* in cowpea seeds treated with botanicals

Table 2: Mortality of *C. maculatus* in stored cowpea seeds due to botanicals

Treatment	Corrected (% adult mortality of <i>C. maculatus</i> after monthly intervals									
	1 Month	2 Month	3 Month	4 Month	5 Month	6 Month	7 Month	8 Month	9 Month	Pooled
Neem seed kernel powder	83.02 ^{ab} (98.48)	80.03 ^a (96.96)	72.56 ^{bc} (90.88)	66.80 ^{ab} (84.31)	61.94 ^{ab} (77.70)	53.49 ^{bc} (64.55)	42.60 ^b (45.82)	34.99 ^b (32.91)	23.52 ^{bc} (15.95)	57.49 ^c (67.50)
Neem leaf powder	80.03 ^c (96.96)	77.11 ^b (94.94)	62.91 ^d (79.24)	58.49 ^d (72.66)	53.48 ^d (64.56)	47.25 ^e (53.92)	33.60 ^{ef} (30.63)	28.99 ^{de} (23.55)	15.68 ^e (7.34)	51.08 ^{ef} (58.20)
Mint leaves powder	79.69 ^c (96.71)	74.03 ^c (92.40)	62.24 ^d (78.23)	57.55 ^d (71.14)	52.41 ^d (62.79)	45.80 ^e (51.39)	32.97 ^f (29.62)	26.86 ^e (20.50)	13.87 ^f (5.82)	49.58 ^f (56.51)
Custard apple seed powder	80.09 ^{bc} (97.47)	78.83 ^{ab} (96.20)	64.45 ^d (81.27)	58.89 ^d (73.18)	55.93 ^{cd} (68.60)	48.58 ^{de} (56.21)	36.38 ^{de} (35.19)	30.37 ^{cd} (25.57)	16.78 ^e (8.35)	52.15 ^e (60.23)
Neem oil	83.02 ^{ab} (98.48)	80.49 ^a (97.21)	76.31 ^{ab} (94.18)	68.46 ^a (86.32)	62.75 ^{ab} (78.73)	55.63 ^{ab} (68.09)	46.96 ^a (53.41)	37.58 ^a (37.21)	24.30 ^b (16.96)	59.46 ^b (70.07)
Castor oil	81.35 ^{bc} (97.72)	79.57 ^{ab} (96.71)	69.36 ^c (87.33)	63.27 ^{bc} (79.75)	60.17 ^b (75.19)	51.06 ^{cd} (60.50)	39.67 ^c (40.76)	33.59 ^b (30.63)	22.30 ^c (14.42)	55.30 ^d (64.78)
Karanj oil	81.33 ^{bc} (97.71)	78.94 ^{ab} (96.20)	65.32 ^d (82.53)	61.53 ^{cd} (77.21)	59.15 ^{bc} (73.68)	50.77 ^{cd} (59.99)	38.73 ^{cd} (39.24)	32.65 ^{bc} (29.11)	18.78 ^d (10.38)	54.16 ^d (62.89)
Azadirachtin 10000 ppm	83.62 ^a (98.73)	80.89 ^a (97.47)	78.97 ^a (96.20)	70.60 ^a (88.86)	65.53 ^a (82.78)	57.56 ^a (71.15)	49.59 ^a (57.98)	39.38 ^a (40.25)	27.99 ^a (22.03)	61.46 ^a (72.83)
Mean	81.74 (97.94)	78.38 (95.76)	69.01 (86.23)	63.20 (79.18)	58.92 (73.00)	51.03 (60.72)	40.06 (41.58)	33.05 (29.97)	20.40 (12.66)	55.08 (64.13)
S.Em. \pm Treatment(T)	0.71	0.80	1.34	1.36	1.34	1.09	0.99	0.82	0.61	0.63
Period (P)	-	-	-	-	-	-	-	-	-	0.36
T \times P	-	-	-	-	-	-	-	-	-	1.03
C.D. at 5% (T)	1.94	2.34	3.92	3.96	3.91	3.19	2.89	2.41	1.79	1.79
P	-	-	-	-	-	-	-	-	-	1.02
T \times P	-	-	-	-	-	-	-	-	-	2.88
C.V.%	1.63	2.04	3.89	4.29	4.54	4.26	4.95	4.99	6.03	3.75

Notes: 1 Means within the parentheses are retransformed values, those outside are \sqrt{X} transformed value
 2 Treatment mean(s) with letter(s) in common are non-significant by DNMRT at 5% level of significance

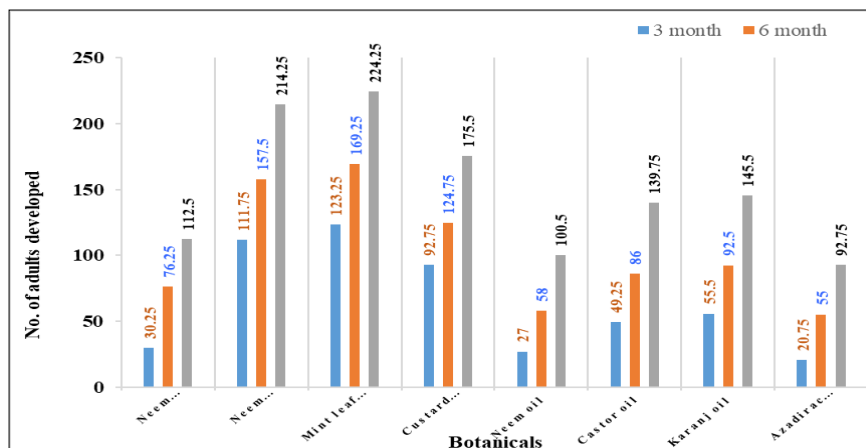


Fig 2: Population growth of *C. maculatus* in cowpea seeds treated with botanicals

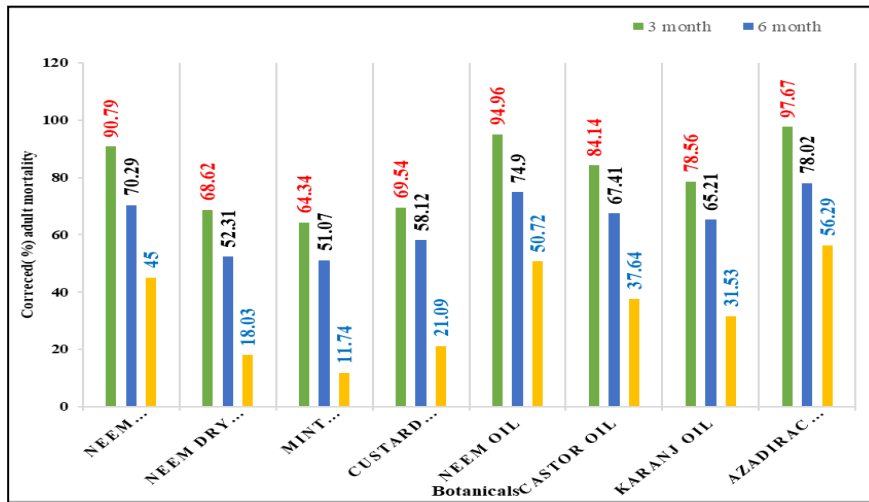


Fig 3: Corrected adult mortality of *C. maculatus* in stored cowpea seeds treated with different botanicals

Table 3: Population growth and adult mortality of *C. maculatus* due to botanicals

Treatments	No. of adults developed after indicated periods of storage *			Corrected (%) adult mortality during indicated period of population **		
	3 months	6 months	9 months	3 months	6 months	9 months
Neem seed kernel powder	5.55 ^b (30.80)	8.69 ^b (75.45)	10.63 ^b (112.95)	72.40 (90.79)	56.98 (70.29)	42.13 (45.00)
Neem leaf powder	10.45 ^e (109.11)	12.53 ^e (157.05)	14.65 ^e (214.66)	56.30 (68.62)	46.33 (52.31)	25.05 (18.03)
Mint leaf powder	11.10 ^e (123.14)	12.92 ^e (166.80)	14.99 ^e (224.71)	53.40 (64.34)	45.61 (51.07)	19.86 (11.74)
Custard apple seed powder	9.59 ^d (92.50)	10.93 ^d (119.38)	13.25 ^d (175.63)	56.61 (69.54)	49.68 (58.12)	27.22 (21.09)
Neem oil	5.08 ^{ab} (25.79)	7.65 ^a (58.48)	10.05 ^a (100.94)	77.33 (94.96)	58.42 (72.51)	44.53 (49.18)
Castor oil	6.92 ^c (47.81)	9.26 ^c (85.80)	11.84 ^{cd} (140.17)	66.59 (84.14)	55.20 (67.41)	37.84 (37.64)
Karanj oil	7.38 ^c (54.46)	9.54 ^{cd} (91.05)	12.08 ^d (145.96)	62.55 (78.56)	53.87 (65.21)	34.14 (31.53)
Azadirachtin 10000 ppm	4.63 ^a (21.42)	7.29 ^a (53.15)	9.66 ^a (93.24)	81.40 (97.67)	62.08 (78.02)	48.62 (56.29)
Control	15.32 ^f (234.72)	19.55 ^f (380.09)	27.20 ^f (739.73)	-	-	-
Mean	8.40 (80.53)	11.01 (129.07)	13.84 (191.46)	65.82 (81.08)	53.52 (64.37)	34.92 (33.81)
S. Em. ±	0.16	0.17	0.16	2.10	0.89	1.20
C.D. at 5 %	0.47	0.49	0.47	6.12	2.61	3.51
C.V. %	3.82	4.11	2.36	6.37	3.91	6.88

Note: 1. Mean in parentheses are retransformed values, those outside are \sqrt{X} * and arc sin ** transformed values
 2. Treatment mean(s) with letter(s) in common are non-significant by DNMRT at 5% level of significance

Conclusions

- All the tested botanicals significantly increased adult mortality of *Callosobruchus maculatus* (F.) compared to the control, achieved over 90% mortality for up to one month. Azadirachtin @ 1.5 ml/kg, neem seed kernel powder @5 g/kg and neem oil @5 ml/kg sustained mortality above 80% for up to four month, while other botanicals were effective for only three months.
- It is evident from this sequence that the population growth was highest in the untreated control, indicating significant protection offered by all botanical treatments. Among the botanicals, azadirachtin @ 1.5 ml/kg (92.75), neem oil @ 5 ml/kg (100.50), and neem seed kernel powder @ 5 g/kg (112.50) proved to be the most effective, recording the least population growth of the pulse beetle.
- Based on the adult mortality of *Callosobruchus maculatus* (F.) recorded after three, six, and nine months of cowpea storage, it was evident that all botanical treatments were effective in suppressing the population growth of the test insect compared to the control.
- However, the degree of efficacy varied among the treatments. Azadirachtin was found to be the most effective, followed by neem seed kernel powder and neem oil.

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