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Effect of Botanical Powders on Controllng Pulse Beetle (*Callosobruchus maculatus* Fabricius) in Cowpea (*Vigna unguiculata*) Seeds Under Laboratory Conditions

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ABSTRACT

Purpose: *Adverse impacts of the application of synthetic pesticides, especially in stored grains, necessitate considering alternate and safe methods for managing pests such as Callosobruchus maculatus in warehouses. Therefore, the potential of various indigenous plant extracts was tested against C. maculatus under laboratory conditions.*

Research Method: *Leaves of Azadirachta indica and Mentha longifolia, fruits of Citrullus colocynthus, Allium sativum, and Trigonella foenum-graecum seeds at a rate of 2 grams in 100 grams of cowpeas along with aluminum phosphine tablets were applied in separate jars that contained 5 fresh pairs of C. maculatus. Observations were taken at 24 hours, 48 hours, and one week for the toxicity of botanicals, whereas the population of beetles was also recorded monthly for three months.*

Findings: *All botanicals were found to be effective in causing mortality of C. maculatus adults. M. longifolia, A. indica, and C. colocynthus caused 100% mortality of targeted C. maculatus within one week, whereas aluminum phosphine took only 24 hours to kill all beetles with no further population development. A gradual rise in C. maculatus population was recorded in T. foenum-graecum and A. sativum treatments, whereas a slow growth was recorded in M. longifolia and A. indica. The highest population and maximum weight loss were recorded in the control, whereas M. longifolia and A. indica treatments suffered lower losses.*

Originality/value: *All the botanicals were found effective against C. maculatus, with M. longifolia and A. indica being the most effective; hence, they may be applied for its management in warehouses.*

Keywords: *Botanical powder, Callosobruchus maculatus, stored cowpea grains.*

INTRODUCTION

Callosobruchus maculatus F. (Coleoptera: Chrysomelidae) is considered one of the most damaging insect pests of stored pulses with estimated losses of 90% within 6 months of its infestation (Bibi *et al.*, 2021; Agour *et al.*, 2022). Considering its huge damage to pulses, mostly synthetic pesticides are used to manage *C. maculatus* populations in the stored pulses (Kedia *et al.*, 2015). However, these pesticides adversely affect humans and non-target organisms and can pollute soil and water (Mahanta *et al.*, 2021; Rani *et al.*, 2021).

Therefore, various alternatives have been evaluated against *C. maculatus* (Xie *et al.*, 2020) in various regions of the world that include the use of high temperatures and UV-light radiation (Kalpna *et al.*, 2022) along with various phytochemicals (botanicals) that have long been used for the management of devastating insect pests, especially in the warehouses (Basile *et al.*, 2022).

Accordingly, in recent years, plant extracts along with secondary metabolites of plants have been

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getting popularity as potential alternatives to synthetic pesticides in managing pest populations (Batiha *et al.*, 2021). The biopesticides have been found to be very effective and target specific pests with a minimum potential for developing resistance among pest populations as well as have low hazardous to the environment (Tozlu *et al.*, 2011). Among botanicals, neem (*Azadirachta indica*) has been successfully used in various regions of the world against a variety of stored grain pests, including *C. maculatus* (Radha and Susheela, 2014; Saxena and Sayyed, 2018; Akbar *et al.*, 2022a, b). Many other indigenous plant extracts, i.e., *Erucas sativa*, *Brassica nigra*, *Allium sativum*, *Curcuma longa*, *Citrus* spp., and *Eucalyptus* sp., have been evaluated against *C. maculatus* in different countries based on their easy availability and preparation (Saxena and Sayyed, 2018; Fouad *et al.*, 2020; Nizamani *et al.*, 2020; Bibi *et al.*, 2021; Ahmed *et al.*, 2022; Madavi *et al.*, 2022).

Therefore, considering the effectiveness and easy availability of botanicals in managing *C. maculatus* in various regions of the world, studies were carried out to determine the potential of various locally available botanicals for their insecticidal performance against *C. maculatus* under laboratory conditions.

MATERIALS AND METHODS

Collection and Preparation of Botanical Extracts

Fresh neem leaves (*Azadirachta indica* Linn.), bitter apple fruits (*Citrullus colocynthus* Linn.), fenugreek seeds (*Trigonella foenum-graecum*), garlic fruits (*Allium sativum* Linn.), and mint leaves (*Mentha longifolia* Linn.) were collected from the local markets or vicinities of the Sindh Agriculture University, Tandojam, Pakistan. After collection, plant materials were air-dried in the laboratory for seven days until completely dried and then ground using a GEEPAS model electric grinder (China GCG289). The obtained powder was sieved through a fine muslin cloth with a pore size of 2 mm to get fine powders, which were stored in separate clean plastic jars with proper labeling.

Experimental Design, Data Collection, and Analysis

Each botanical powder was applied at the rate of 2%, i.e., 2 grams into 100 grams of cowpea seeds (*Vigna unguiculata* var. Rawan-2003) in separate plastic transparent jars of 300 mL to observe their effect on the mortality and population fluctuation of *C. maculatus*. After application, each botanical treatment was shaken thoroughly with cowpea seeds for three minutes to create a homogenous mixture in the jar. In addition to botanical powder treatments, two control treatments were also used: a positive control using aluminum phosphine tablets applied at its recommended dose (0.25 grams per square foot), and a negative control (without any botanical insecticide).

After treatment application, five freshly emerged pairs (10 individual insects: five males and five females) of *C. maculatus* were released into individual jars, which were covered with muslin cloth and secured with elastic rubber bands to ensure aeration and containment of the insects. All treatments were placed in separate plastic cages of 2 cubic square feet to avoid cross-treatment interference, particularly from aluminum phosphine tablets. The moisture content of cowpea seeds in all treatments was adjusted to 12% before insect release and treatment application.

The experiment followed a Completely Randomized Design (CRD) with five replications. Toxicity of botanical powders against *C. maculatus* was recorded at 24 hours, 48 hours, and 7 days post-application. Population data of live and dead adult *C. maculatus* were collected monthly for three consecutive months. At the end of the third month, 100 seeds from each treatment replication were randomly sampled to determine the number of damaged and undamaged grains. Additionally, seed weight was measured to assess weight loss due to feeding activity of *C. maculatus*.

The following formulas were used:

$$\%\text{ damaged grains} = \left(\frac{\text{damaged grains}}{\text{total grains}}\right) \times 100$$

$$\%\text{WL} = \left(\frac{\text{FWG}}{\text{IWG}}\right) \times 100$$

WL = Weight loss; FWG = Final weight of grains; IWG = Initial weight of grains.

A separate experiment was conducted to assess the impact of the above treatments on the germination capability of cowpea seeds. Ninety days post-treatment, ten seeds from each replicated treatment were randomly selected and placed on moist filter paper in petri dishes. These were kept for seven days at ambient laboratory conditions ($30 \pm 2\,^{\circ}\text{C}$) temperature and ($60 \pm 5\%$) relative humidity). Germinated seeds were counted, and germination percentage was determined accordingly.

Statistical Analysis

Analysis of Variance (ANOVA) was employed for data analysis. Means showing significant differences were separated using the Least Significant Difference (LSD) test at a 5% probability level. The software STATISTIX 8.1 was used for performing statistical analysis.

RESULTS AND DISCUSSION

It was observed in the study that all the botanical powders used were effective in causing mortality among adults of *C. maculatus*, as a rise in adult mortality was recorded over time (Fig.01). The percentage mortality of *C. maculatus* recorded 24 hours after the application of botanical powders, aluminum phosphine, and control treatments showed a highly significant difference ($F = 42.70$, $P < 0.001$) among the treatments. All treated adults were found dead in the aluminum phosphine treatment (positive control), whereas no mortality was recorded in the control (negative control). Among botanical powders, the highest mortality percentage ($68.00 \pm 5.83\%$) of adult *C. maculatus* was recorded in *M. longifolia*, but it was not significantly different from the mortality recorded in *A. indica* ($60.00 \pm 7.07\%$), *C. colocynthus* ($58.00 \pm 3.74\%$), and *T. foenum-graecum* ($56.00 \pm 5.10\%$) treatments. Significantly, the lowest mortality percentage after 24 hours of exposure was recorded in *A. sativum* ($50.00 \pm 4.47\%$) (Fig.01).

A rise in adult mortality was further observed after 48 hours and 7 days of the application of botanical powders, with significant differences among treatments ($F = 53.30$, $P < 0.001$ and $F = 277.00$, $P < 0.001$, respectively) (Fig.01). After 48 hours, although 100% mortality was observed in the aluminum phosphine treatment, it was not significantly different from the mortality recorded in *M. longifolia* ($90.00 \pm 4.47\%$). The mortality percentages in *A. indica* ($82.00 \pm 6.63\%$), *A. sativum* ($80.00 \pm 4.47\%$), and *C. colocynthus* ($78.00 \pm 3.74\%$) were also not significantly different from that of *M. longifolia*. The lowest mortality ($72.00 \pm 5.83\%$) was observed in *T. foenum-graecum*, but it was also not significantly different from the above-mentioned three treatments.

After 7 days, 100% mortality of adult *C. maculatus* was recorded in the aluminum phosphine, *M. longifolia*, *A. indica*, and *C. colocynthus* treatments. Significantly different mortality percentages were recorded in *A. sativum*, *T. foenum-graecum*, and control treatments as $90.00 \pm 3.16\%$, $82.00 \pm 3.74\%$, and $6.00 \pm 2.45\%$, respectively (Fig.01).

The application of botanical powders and aluminum phosphate not only caused significant mortality of *C. maculatus* adults but also elicited a substantial impact on its further population development (Fig.02). According to the results, no alive adult *C. maculatus* was observed in any of the treatments (including control) after one month of observation; that may be due to the mortality of released beetles either due to the application of various treatments or their short adult longevity. Moreover, no population of adult beetles was recorded in the aluminum phosphine treatment during the entire study duration, whereas a rapid increase in the beetle population was recorded in the negative control treatment, especially during the 3^{rd} month after the application of various treatments (Fig.02).

A gradual increase in the population of adult alive *C. maculatus* was recorded in various botanical powder treatments, especially *T. foenum-graecum* and *A. sativum*, whereas a slow increase in the alive population was recorded in *M. longifolia* and *A. indica* treatments. Accordingly, the highest mean population after the 2^{nd} and 3^{rd} months of observations was recorded in the negative control (55.80 ± 7.10 and 656.80 ± 32.82 beetles, respectively). Among botanical powder treatments, the highest mean population of alive *C. maculatus* after the 2^{nd} and 3^{rd} months was recorded in *T. foenum-graecum* (27.80 ± 3.65 and 99.40 ± 8.21 beetles, respectively), but the same was not significantly different from the population observed in *A. sativum* treatments with a mean population of 20.20 ± 6.42 and 58.40 ± 11.67 beetles, respectively.

Overall, the minimum adult population of alive *C. maculatus* at the end of the 2^{nd} and 3^{rd} months of observation was recorded in *M. longifolia* with a mean population of 4.80 ± 1.07 and 28.60 ± 6.84 beetles, respectively, but the same were not significantly different from the mean population recorded in the *A. indica* treatment with a mean population of 11.60 ± 2.56 and 47.60 ± 9.11 beetles, respectively. Thus, a highly significant difference was recorded among treatments with respect to the population of alive adult *C. maculatus* after the 2^{nd} ($F = 19.00$, $P < 0.001$) and 3^{rd} ($F = 254.00$, $P < 0.001$) months of their application. Figure 03 shows the results of the effect of botanical powders along with aluminum phosphine (positive control) and control (negative control) treatments on the monthly mean population of dead adult *C. maculatus*. The results confirmed a significant difference in the mean population of dead *C. maculatus* after the 1^{st} ($F = 391.00$, $P < 0.001$), 2^{nd} ($F = 3.43$, $P = 0.0115$), and 3^{rd} ($F = 16.50$, $P < 0.001$) months of observations. At the end of the

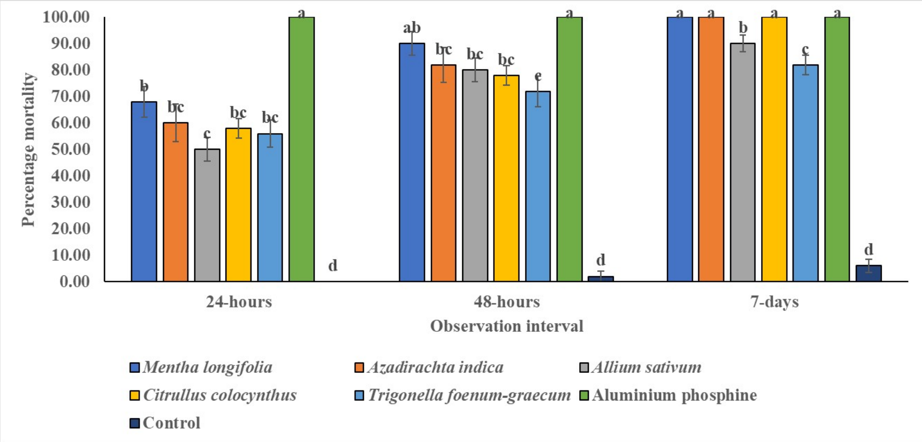

Figure 1: Effect of selected botanical powders on the mortality percentage of *Callosobruchus maculatus* adults in cowpea seeds under laboratory conditions.
**Means followed by same letters against each observation interval are not significantly different from each other at $P < 0.05$ (LSD values: 24-hours = 13.139, 48-hours = 12.769, 7-days = 5.9971).*

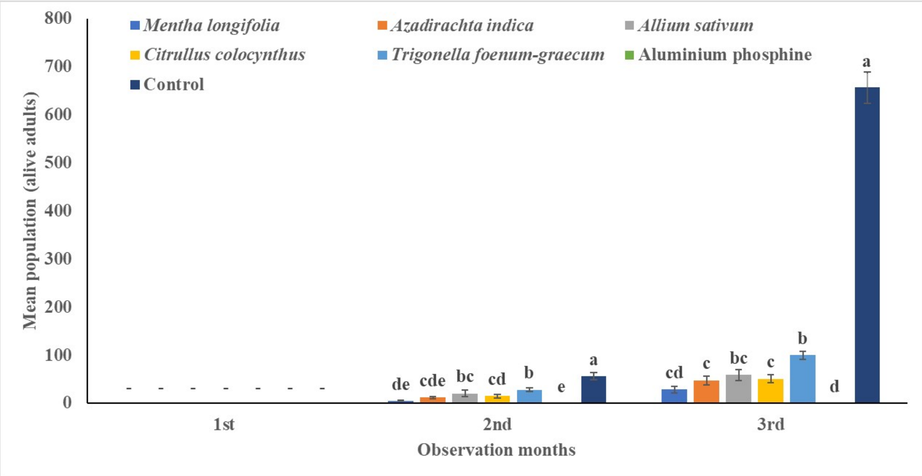

Figure 2: Effect of botanical powders on monthly mean population of alive *Callosobruchus maculatus* adults in cowpea seeds.
**Means followed by same letters against each observation intervals are not significantly different from each other at $P < 0.05$ (LSD values 2-months = 12.323, 3-months = 42.201.*

1st month, the dead adults were only recorded in the control (9.40 ± 0.24 beetles) and *T. foenum-graecum* (1.40 ± 0.40 beetles). Moreover, the highest and lowest mean populations of dead *C. maculatus* adults after two months of application of various treatments were recorded in *T. foenum-graecum* (4.00 ± 0.71 beetles) and *A. sativum* (2.20±0.58 beetles) treatments. A rapid increase in the population of dead *C. maculatus* adults was recorded during the 3rd month in cowpeas. The significantly highest mean population was recorded in the negative control (55.20 ± 7.36 beetles), followed by *T. foenum-graecum* (35.80 ± 4.39 beetles). The significantly lowest dead adults after three months were recorded in *M. longifolia* (8.00 ± 1.73 beetles), but the same was not significantly different from the dead adult population recorded in the *A. indica* (12.80 ± 2.48 beetles) treatment. Results of the application of botanical powders along with aluminum phosphine and negative control on the percentage damage of cowpea seeds by *C. maculatus* are given in Figure 04. The application of botanical powders caused a significant reduction in the damage by *C. maculatus* in cowpea seeds, as all the treatments suffered significantly lower losses compared to the negative control treatment. According to the results, cowpea seeds in the negative control treatment suffered significantly the highest damage by *C. maculatus* with respect to the percentage of damaged grains (100%) and weight loss (89.80±2.06%), whereas no damage to cowpea seeds was recorded in the aluminum phosphine treatment. Among botanical powder treatments, the lowest percentage of damaged grains (7.40 ± 1.29%) and weight loss (4.80 ± 0.97%) was recorded in *M. longifolia*, but it was not significantly different from *A. indica* with a percentage of damaged grains and weight loss of 9.40 ± 1.60% and 7.60 ± 1.03%, respectively.

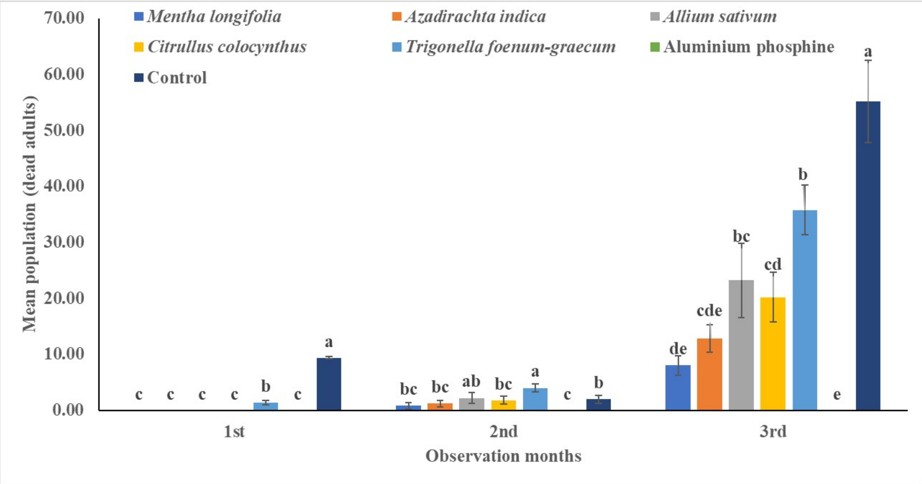

Figure 3: Effect of botanical powders on monthly mean population of dead *Callosobruchus maculatus* adults in cowpea seeds .
**Means followed by same letters against each observation intervals are not significantly different from each other at* $P < 0.05$ *(LSD values 1-month = 0.5136, 2-months = 1.9769, 3-months = 13.239).*

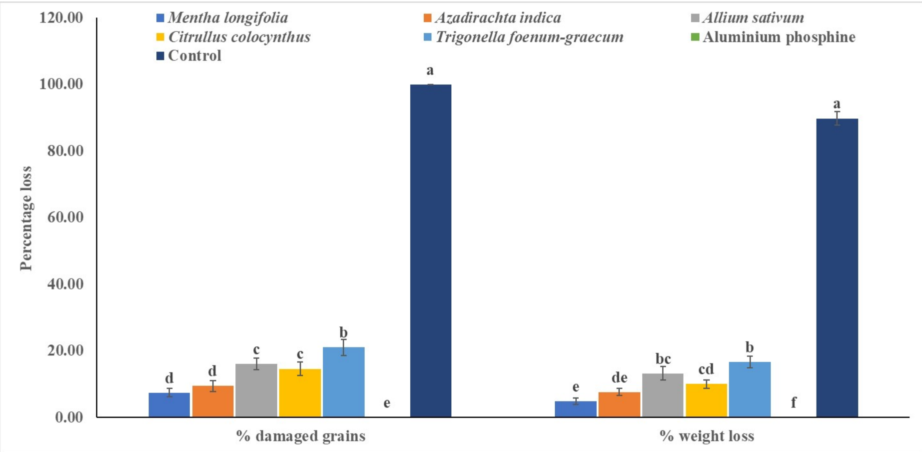

Figure 4: Impact of various botanical powders on percentage loss of cowpea seeds by *Callosobruchus maculatus* .
** Means followed by same letters in each category are not significantly different from each other at* $P < 0.05$ *(LSD value: % damage grains = 4.5118, % weight loss = 4.2179)*

The percentage of damaged grains recorded in the remaining treatments, i.e., *C. colocynthis*, *A. sativum*, and *T. foenum-graecum*, were $14.60 \pm 2.06\%$, $16.00 \pm 1.70\%$, and $21.00 \pm 2.37\%$, respectively, whereas the percentage of weight loss in the same treatments was observed as $10.00 \pm 1.22\%$, $13.20 \pm 2.03\%$, and $16.60 \pm 1.72\%$, respectively. Thus, statistically, a significant difference was recorded among various botanical powder treatments and control treatments with respect to the percentage of damaged cowpea seeds ($F = 481.0$, $P < 0.001$) and weight loss ($F = 457.0$, $P < 0.001$). Results regarding the effect of various botanicals along with aluminum phosphate and control treatments on the germination percentage of cowpea seeds have been given in Figure 05. No significant ($F = 1.00$, $P = 0.448$) effect of the application of various treatments was recorded on the germination capability of the cowpeas. According to the results, 100% germination of cowpea seeds was recorded in the control and aluminum phosphate treatments, whereas germination percentages recorded among other treatments, i.e., *M. longifolia*, *A. indica*, *A. sativum*, *C. colocynthis*, and *T. foenum-graecum*, were $97.00 \pm 2.00\%$, $98.00 \pm 2.00\%$, $96.00 \pm 2.45\%$, $99.00 \pm 1.00\%$, and $96.00 \pm$.

All the botanicals used in the study, i.e., *A. indica*, *C. colocynthis*, *T. foenum-graecum*, *A. sativum*, and *M. longifolia*, were found effective against *C. maculatus*, as all of these not only cause mortality of the adults but also cause significant reduction in its further population development. However, there was a significant difference among botanical powders and synthetic aluminum phosphine tablets used in the study, as aluminum phosphine caused 100% mortality of the *C. maculatus* adults with

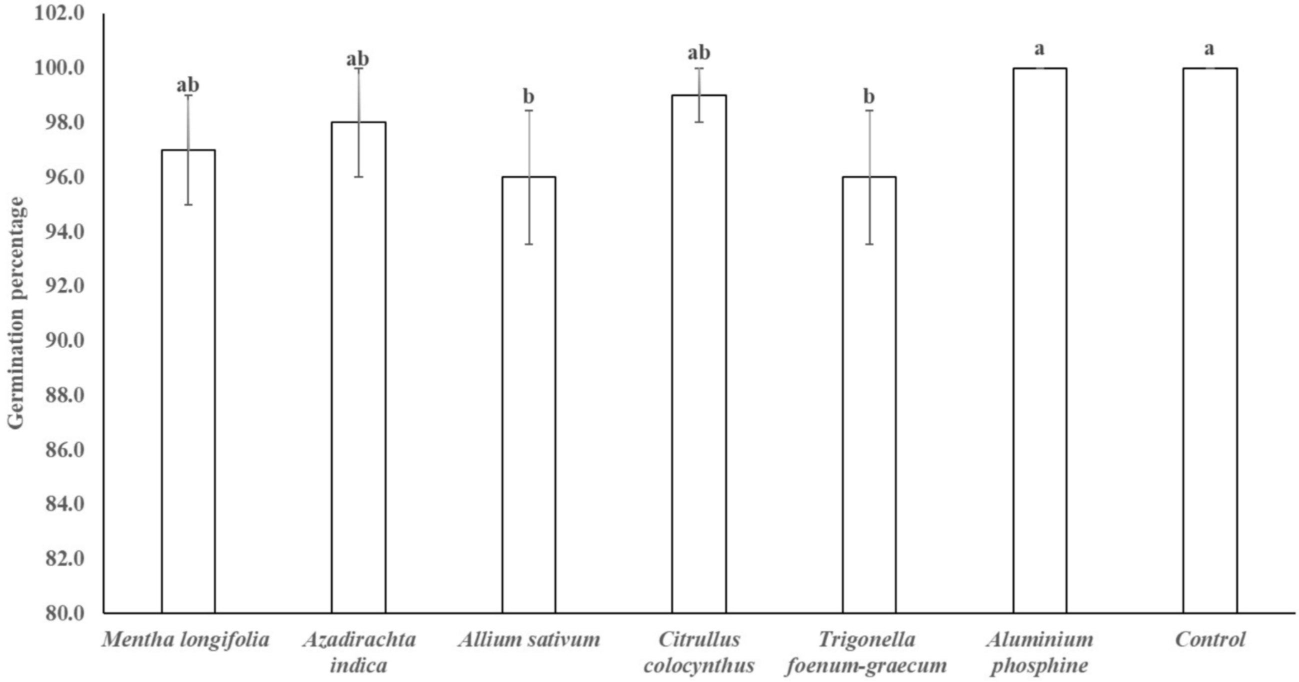

Figure 5: Impact of various botanical powders used against *Callosobruchus maculatus* on germination percentage of cowpea seeds .
** Means followed by same letters are not significantly different from each other at (LSD = 5.0176,* $P < 0.05$*)*

no further population development in the treatment. Among botanical powders, *M. longifolia* and *A. indica* were found to be the most effective with respect to mortality and population reduction of *C. maculatus*, whereas *T. foenum-graecum* and *A. sativum* were found comparatively less effective. Considering the target specificity and less hazardous effect of botanicals, their use has shown an increasing trend in agriculture, especially against stored grain pests, including *Callosobruchus* spp. Therefore, a large number of plant powders, extracts, and essential oils have been used either as repellents or insecticides against a huge range of stored grain pests. Among the botanicals, *A. indica* plant extracts and neem products are widely used against dangerous insect pests, both in the field and in the warehouse, since ancient times, mainly because of their wide range of actions against target pests.

A recent study evaluating the repellency of five botanicals against *C. maculatus* also found *A. indica* to be the most effective repellent, followed by *Melia azedarach*, whereas *Nicotiana tabacum*, *N. rustica*, and *Thuja orientalis* were comparatively less repellent for *C. maculatus* adults. However, Akbar *et al.* (2022a) recorded 80.56% mortality of *C. maculatus* with *A. indica* extracts, whereas 100% and 86.11% mortality was recorded with the application of 3% (v/v) extracts of *N. tabacum* and *N. rustica*, respectively. Saxena and Sayyed (2018) also found that extracts of *A. indica*, *A. sativum*, *Curcuma longa*, *Citrus sinensis*, *Citrus limon*, *Murraya koenigii*, and *Eucalyptus* sp. have the repellent and insecticidal potential against *C. maculatus*. Among these botanicals, *A. indica* caused maximum mortality (58%), followed by *A. sativum* (52.66%) and *C. longa* (49.33%), whereas *A. indica* also exhibited a maximum repellency index (IR) value of 0.53, followed by *A. sativum* (IR = 0.66). The results of the above-mentioned studies supported our findings, as besides *M. longifolia*, *A. indica* also caused 100% mortality of *C. maculatus* within 7 days of the exposure. Moreover, both these treatments, i.e., *M. longifolia* and *A. indica*, caused significant reduction in the future population development and damage of *C. maculatus*. Considerable reduction in the population of beetles was also recorded by Radha and Susheela (2014) with the application of *A. indica* extracts, as Dubey *et al.* (2008) reported that azadirachtin in *A. indica* is mainly responsible for affecting the feeding, growth, molting, and reproduction of insects.

Accordingly, many other studies also confirmed the insecticidal potential of *A. indica* extracts and oils against a wide variety of stored grain pests, including *Callosobruchus* spp., as their results were found to be at par with the synthetic insecticides.

Among the botanicals, *M. longifolia* was most effective with respect to mortality and future population reduction of *C. maculatus*; hence, cowpea seeds suffered the lowest damage in the treatment that was comparable to synthetic aluminum phosphine. Mirshekar (2021) evaluated the essential oils of *M. longifolia* and *M. mozaffarianii* against *C. maculatus* and found that *M. mozaffarianii* essential oil was comparatively more toxic and repellent than *M. longifolia* with LC_{50} values after 96 hours of 55.4 and 38.5 µL/L, respectively. The piperitenone oxide-type *M. longifolia* essential oil and a 10% (w/w) nano-emulsion of its essential oil were tested against various stored grain pests of wheat, i.e., *T. castaneum*, *T. confusum*, *Tenebrio molitor* L., *Oryzaephilus surinamensis* L., and *Acarus siro* L. The results suggested that both treatments were found effective to cause significant mortality of all the targeted pest species, as almost 100% mortality was recorded for both larvae and adults. Shafaie *et al.* (2019a) also confirmed comparatively more mortality of *C. maculatus* than *S. granaries* with the application of *M. longifolia* essential. Moreover, the insecticidal potential of *M. longifolia* extracts and essential oils has also been confirmed against many noxious stored grain pests that include *T. castaneum*, *T. confusum*, *Sitophilus granaries*, and *O. surinamensis* (Shafaie *et al.*, 2019b; Bhatti *et al.*, 2020; Memon *et al.*, 2020). Moreover, most of these studies also confirmed that the application of *M. longifolia* in stored grains could result in lower grain damage by the pests. Therefore, the insecticidal effectiveness of *M. longifolia* against many stored grain pests, including *C. maculatus*, supported the findings of our study, as its application not only caused 100% mortality of adult *C. maculatus* within seven days of exposure but also the lowest grain damage and weight of cowpea seeds was also observed in this treatment, which was not significantly different from the commercial application of aluminum phosphine tablets by commercial warehouse managers.

Besides, *A. indica* and *A. sativum* were also found to be effective botanicals in our study that caused 90% adult mortality and a considerable reduction in the number of *C. maculatus* and reduced cowpea seed damage. Studies of Boeke *et al.* (2004), Kosini *et al.* (2015), and Nukenine *et al.* (2010) also reported that *A. sativum* has insecticidal potential against *C. maculatus*, not only causing the death of the targeted beetles but also proving to be repellent with less population growth. *Allium sativum* mainly affects the midgut of target insects due to the presence of lectins in it, leading to their death (Upadhyay and Singh, 2012).

It was also observed in the study that the application of various botanical insecticides in cowpea seeds against *C. maculatus* did have any significant impact on their germination capability. Accordingly, the germination of cowpea seeds recorded in treatments ranged between 96% and 100%, showing no significant variation among themselves. Talib (2020) also found no significant impact of the application of three vegetable oils (*Cinnamomum cassia*, *Piper cubeba*, and *Mentha* spp.) along with untreated control against *C. maculatus* on the germination capability of mung bean seeds, as germination was recorded in the range of 77.5 to 87.5%. However, Sharma *et al.* (2016b) found a significant reduction in the germination of pigeon pea as compared to 96% germination in untreated control; the seed germination recorded in neem essential oil was 88.66%. The reduced germination percentage of treated seeds recorded in this study in comparison to our study may be because we used botanicals as powders, whereas Sharma *et al.* (2016a) used neem essential oils. However, Asawalam and Anaeto (2014) observed significantly more germination of cowpea seeds treated with various botanicals, including *A. sativum*, than in the control treatment infested with *C. maculatus*. Similarly, a significant effect of botanicals was recorded on the germination of chickpea seeds as compared to the control; a higher germination percentage was recorded in *Datura stramonium*, *A. indica*, and *A. sativum* treatments. Overall, the application of botanical insecticides, particularly in the shape of powder extracts, not only reduces the infestation of *C. maculatus* but also does not exhibit any negative impact on the germination capability of the pulse grains.

CONCLUSIONS

All the botanical insecticides used in the study were found effective to cause mortality of *C. maculatus* adults and reduce its further population growth as compared to growth; however, they varied in their effectiveness. *Mentha longifolia* and *A. indica* were found to be the most effective botanicals, as their performance with respect to mortality, population growth, and reduction in percentage damage was comparable with the synthetic aluminum phosphine tablets used in the study. The minimum percentage of damaged grains and weight loss was also recorded in *M. longifolia* and *A. indica* treatments, whereas untreated controls suffered maximum beetle population, percentage of damaged grains, and weight loss.

Moreover, no significant difference in the germination percentage of cowpea seeds was recorded among various treatments. Therefore, it is suggested that the application of *M. longifolia* and *A. indica* powders may be evaluated in the warehouses to protect the stored pulses from *C. maculatus* that may also lower the adverse effects of the synthetic pesticides.

Declaration of interest statement

Authors declared no conflict of interest.

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