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EFFECT OF FIVE MEDICINAL PLANT LEAVES ON SURVIVAL, OVIPOSITION, AND PROGENY DEVELOPMENT OF COWPEA BRUCHID, C*allosobruchus maculatus* (F.) INFESTING COWPEA SEEDS IN STORAGE

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ABSTRACT

Over 90% of the insect damage to cowpea seeds is caused by *Callosobruchus maculatus*. Although, many chemicals have proven their efficacy against *C. maculatus*, they adversely affect the natural environment. Death of non-targeted species which are natural enemies of other insect pests and development of resistance to chemicals is possible. Therefore, the current study was conducted to determine the effect of plant leaves of *Murraya koenigii* ("Karapincha"), *Pandanus amaryllifolius* ("Rampe"), *Vitex negundo* ("Nika"), *Pavetta indica* ("Pavatta") and *Croton aromaticus* ("Wel keppetiya") on adult mortality, oviposition, and progeny development of *C. maculatus*. Plant leaves were tested in both fresh form and powdered form. The plant leaves were mixed with cowpea seeds to have two concentrations followed by the introduction of 5 pairs of *C. maculatus* adults. The maximum adult mortality, minimum oviposition and the minimum progeny development were observed when the cowpea seeds were treated with *C. aromaticus* leaves. The mortality increased to 100% when the seeds were treated with the combination of *C. aromaticus* – *P. amaryllifolius* – *V. negundo* (WPN) plant leaf powders. This study suggests that fresh plant leaves or plant leaf powder of *C. aromaticus* can be used against *C. maculatus* to protect cowpea seeds in storage.

Keywords: Adult mortality, C*allosobruchus maculatus*, *Croton aromaticus*, Oviposition, Progeny development

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INTRODUCTION

Cowpea, *Vigna unguiculata* is one of the most important food and forage legumes in the semi-arid tropics which includes parts of Asia, Africa, Southern Europe, Southern United States, and Central and South America (Singh, 1977; Timko *et al*., 2007). It belongs to the family Leguminosae, subfamily Papilionaceae and tribe Phaseolae. The dry seeds consist of about 25% protein and 67% carbohydrates. It is also a good source of calcium, iron, vitamins, and carotene (Adedire *et al*., 2011). Thus, it is the cheapest source of dietary protein for most people in many developing countries where per capita daily intake of animal protein is low (Okigbo, 1978; Oparaeke *et al.*, 1998).

Both quantitative and qualitative losses due to physical, chemical, and biological factors including insects during the storage of grains have been reported (Adedire & Akinneye, 2004). *Callosobruchus* species are major pests of stored grains and grain products in the tropics (Ofuya, 2003). Over 90% of the insect damage to cowpea seeds is by *Callosobruchus maculatus* (Caswell & Akibu, 1980). It is known to infest all grains of a complete cowpea harvest within 3 to 5 months of storage (Singh, 1977). Both larvae and adults are responsible for the damage. Female lays eggs singly and are glued onto the surface of the seeds. The larvae hatch in 4-7 days depending on the temperature of the oviposition site (Howe & Currie, 1964; Osuji, 1982). Upon hatching larvae bore into the seeds and feed the kernel. Pupation takes place within the seed and the emerged adult will escape from the seed making a circular hole in the seed coat. This leads to a reduction in kernel weight and diminished market value due to the presence of insects inside the kernels. Bruchid infestation also decreases the germination potential of the kernel (Maina *et al*., 2006).

However, chemical insecticides have been employed in the control of insect pests and often proved to be very effective. They may be applied as a powder, liquid or fumigant gas formulation depending on the intended use of the pesticide (Bhattacharyya *et al*., 2009).

Many of these chemicals have proven to be very effective against *C. maculatus* damage when applied at the right time, in the right dose and using appropriate techniques. Nevertheless, these chemicals are often unavailable for low-income families in villages and the costs are disproportionately high. Besides, a lack of knowledge about the application may reduce the efficacy of the pesticide and cause perilous situations for appliers and consumers. Despite the success in the control of insect pests using synthetic insecticides, there are several drawbacks such as pollution of the environment, toxic residues on stored grains, unavailability of pesticides at critical periods, development of resistant insect strains, pest resurgence, very expensive and lethal effects on non-target organisms (Adedire *et al*., 2011; Ileke & Oni, 2011).

Natural plant products are an excellent alternative to synthetic insecticides to reduce negative impacts on human health and the environment. They are safe, eco-friendly, and more compatible with the environmental components than synthetic insecticides. (Isman & Machial, 2006). Thus, during the past few decades, the interest in the use of plant products as insecticides has increased (Subramanyam & Roesli, 2000). Some of these plant species possess one or more useful properties such as repellency, anti-feedant, fast knock down, flushing action, biodegradability, and the ability to reduce insect resistance (Olaifa *et al*., 1987). The use of botanicals either alone or in synergy for control of *C. maculatus* in both field and storage has proved effective (Rajapakse & Van Emden, 1997). Therefore, the current study was conducted to determine the effect of five types of plant leaves *Murraya koenigii* (Curry leaf, "Karapincha"), *Pandanus amaryllifolius* (Pandan, "Rampe"), *Vitex negundo* (Chinese chaste tree, "Nika"), *Pavetta indica* ("Pavatta") and *Croton aromaticus* ("Wel keppetiya") on adult mortality, oviposition, and progeny development of *C. maculatus.*

METHODOLOGY

The current study was conducted in the insectary at the Department of Zoology and Environmental Management, University of Kelaniya (6º58'20.91" N; 79º54'52.83" E). A pure population of *C. maculatus* was maintained throughout the period of study under the laboratory conditions (28 ± 2°C and 70 ± 5% RH) (Wet and Dry Bulb Hygrometer, Xinmeng, Hong Kong) using the adult insects collected from infested cowpea seeds at grain markets. The plant leaves *M. koenigii*, *P. amaryllifolius, V. negundo, P. indica* and *C. aromaticus* which were used in this study were obtained in fresh form and free of insecticides from the home gardens in Polgampola in Kegalle district, Sri Lanka. Leaves of these plants were rinsed in clean water, cut into smaller pieces of approximately 1 cm and air-dried for 72 hours. They were ground well using an electric grinder (Mixer Grinder HL7699, Philips, Nalagarh). The ground product was subsequently sieved through a fine mesh (0.01 mm) to obtain a fine powder. The cowpea seeds (*V. unguiculata*) of the "Dhawala" variety were obtained from an agricultural seed farm at Mahailuppallama, Sri Lanka where organic farming is practiced ensuring that the seeds were free of insecticides. All the seeds were kept at -5°C for 7 days to prevent the later emergence of insects hidden inside and after 12 hours these defaunated seeds were placed inside an oven at 40°C for 4 hours to ensure that no organism had remained. Finally, they were air-dried in the laboratory for one hour and stored in air-tight plastic containers. The seeds treated in this manner were used to conduct all the tests.

Effect of contact toxicity of fresh plant leaves and plant powders on adult mortality, oviposition, and progeny development of *Callosobruchus maculatus*

Six experiments were carried out to determine the effect of contact toxicity of (a) fresh plant leaves and (b) dried plant powders on (i) adult mortality (ii) oviposition and (iii) progeny development of *C. maculatus*. For these experiments, 20 g of cowpea seeds were added into

250 mL plastic containers. Two grams of different (a) fresh plant leaves and (b) plant powders of *M. koenigii*, *P. amaryllifolius*, *V. negundo*, *P. indica* and *C. aromaticus* were added to each container separately. Five such containers, 4 of them as the treatment and 1 as the control were prepared for each leaf species in each form. No plant leaves or plant powders were added to the control experiment. Thereafter, 5 pairs of adult *C. maculatus* were introduced into each container. Finally, a total of 25 containers were randomly placed in an insect rearing cage.

(i) Adult mortality

The number of dead adults was recorded for 4 days at every 24-hour interval. Adults were assumed dead when they made no response once probed with a pointed pin. All dead adults were removed from each container once data were recorded.

(ii) Oviposition

The number of eggs laid on treated seeds (Ts) and control seeds (Cs) was carefully recorded after 15 days of the introduction of adults to the containers.

(iii) Progeny development

The containers were kept for further 15 days until the emergence of the F1 generation. The newly emerged adults in each container were counted. The percentage progeny adult emergence was calculated as follows. (Odeyemi & Daramola, 2000).

$$\%\ \text{Progeny development} = \frac{\text{No. of adults emerged}}{\text{No. of eggs laid}} \times 100$$

The same procedure was repeated with 5 g of (a) fresh plant leaves and (b) plant powders.

Effect of contact toxicity of the combination of plant powders on adult mortality, oviposition, and progeny development of *Callosobruchus maculatus*

Different plant powders were mixed in equal amounts in these experiments to prepare a 5 g combination. Combinations of plant powders were "Rampe" (*P. amaryllifolius*) -

"Karapincha" (*M. koenigii*) (RK), "Rampe" (*P. amaryllifolius)* - "Wel Kappettiya" (*C. aromaticu*s) (RW), "Wel Kappetiya" (*C. aromaticu*s) - "Nika" (*V. negundo*) (WN) and "Wel keppetiya" (*C. aromaticu*s) - "Pavatta" (*P. indica*) - "Nika" (*V. negundo*) (WPN). Fifty percent of each plant powder was mixed to prepare 2 plant powder combinations and 33.3% of plant powders from each was mixed to prepare 3 plant powder combination.

R – "Rampe" - (*Pandanus amaryllifolius*)

K – "Karapincha" - (*Murraya koenigii*)

W – "Wel keppetiya" - (*Croton aromaticus*)

N – "Nika" - (*Vitex negundo*)

P – "Pavatta" - (*Pavetta indica*)

Three experiments were carried out using 5 g of plant powder from each combination according to the same procedure which was used to determine the effect of contact toxicity of a single plant powder on adult mortality, oviposition, and progeny development of *C. maculatus.*

Statistical analysis

Statistical analyses were performed using MINITAB 14 (Minitab Inc. 2004). First, data were tested for homogeneity of variance. When variances were homogeneous, one-way ANOVA was performed to determine the effect of contact toxicity of fresh plant leaves, plant powders and combination of plant powders on adult mortality, oviposition, and progeny development of *C. maculatus*. The significance was tested at P = 0.05. Tukey's test was used to determine the differences among sample means for significance.

RESULTS AND DISCUSSION

The insecticidal effect of tested plant species varied with the physical form of the plant leaves used, time of exposure and concentration. A significant difference in adult mortality of *C. maculatus* at 2 g application of different fresh plant leaves was observed after

1 day ($F_{5,18}$=11.44, P=0.00), 2 days ($F_{5,18}$=5.84, P=0.002), 3 days ($F_{5,18}$=8.88, P=0.00) and 4 days ($F_{5,18}$=9.01, P=0.00) of exposure to the treatments (Figure 1a). Similarly, the percentage mortality of *C. maculatus* was significantly affected by the application of 5 g of different fresh plant leaves after 1 day ($F_{5,18}$=25.02, P=0.00), 2 days ($F_{5,18}$=16.71, P=0.002), 3 days ($F_{5,18}$=18.02, P=0.00) and 4 days ($F_{5,18}$=24.33, P=0.00) of exposure to the treatments (Figure 1b). Adult mortality increased with the increase in number of exposure days and concentration despite the type of leaf applied. However, the maximum mortality was observed in seeds treated with both 2 g and 5 g of *C. aromaticus* (65.45% and 83.25%) whereas the minimum mortality was recorded in seeds treated with both 2 g and 5 g of *P. indica* (21.82% and 29.74%, respectively; Figure 1a and 1b). This indicates that the phytochemicals present in *C. aromaticus* fresh plant leaves influence the mortality of *C. maculatus* than the other four plant leaves. Chomchalow (2003) stated that a mixture of fresh ground neem leaves protected mung bean seeds against stored weevils.

Studies have verified that fresh or powder application of *Ocimum sanctum* leaves causes a significant reduction in adult longevity, fecundity, hatching and emergence of *C. maculatus* (Rajapakse, 2006). Similarly, a significant difference was observed in the mean number of eggs laid by *C. maculatus* at 2 g ($F_{5,18}$=655.68, P=0.00) and 5 g ($F_{5,18}$= 909.93, P=0.00) application of different fresh plant leaves (Figure 1c). Moreover, the percentage progeny development showed a significant variation with different types of fresh plant leaves at both 2 g ($F_{5,18}$=78.74, P=0.00) and 5 g ($F_{5,18}$=111.18, P=0.00) concentrations (Figure 1d). However, the maximum number of eggs laid, and the percentage progeny development were observed once the seeds were treated with both 2 g and 5 g concentrations of fresh plant leaves of *P. indica* while the minimum was observed once the seeds were

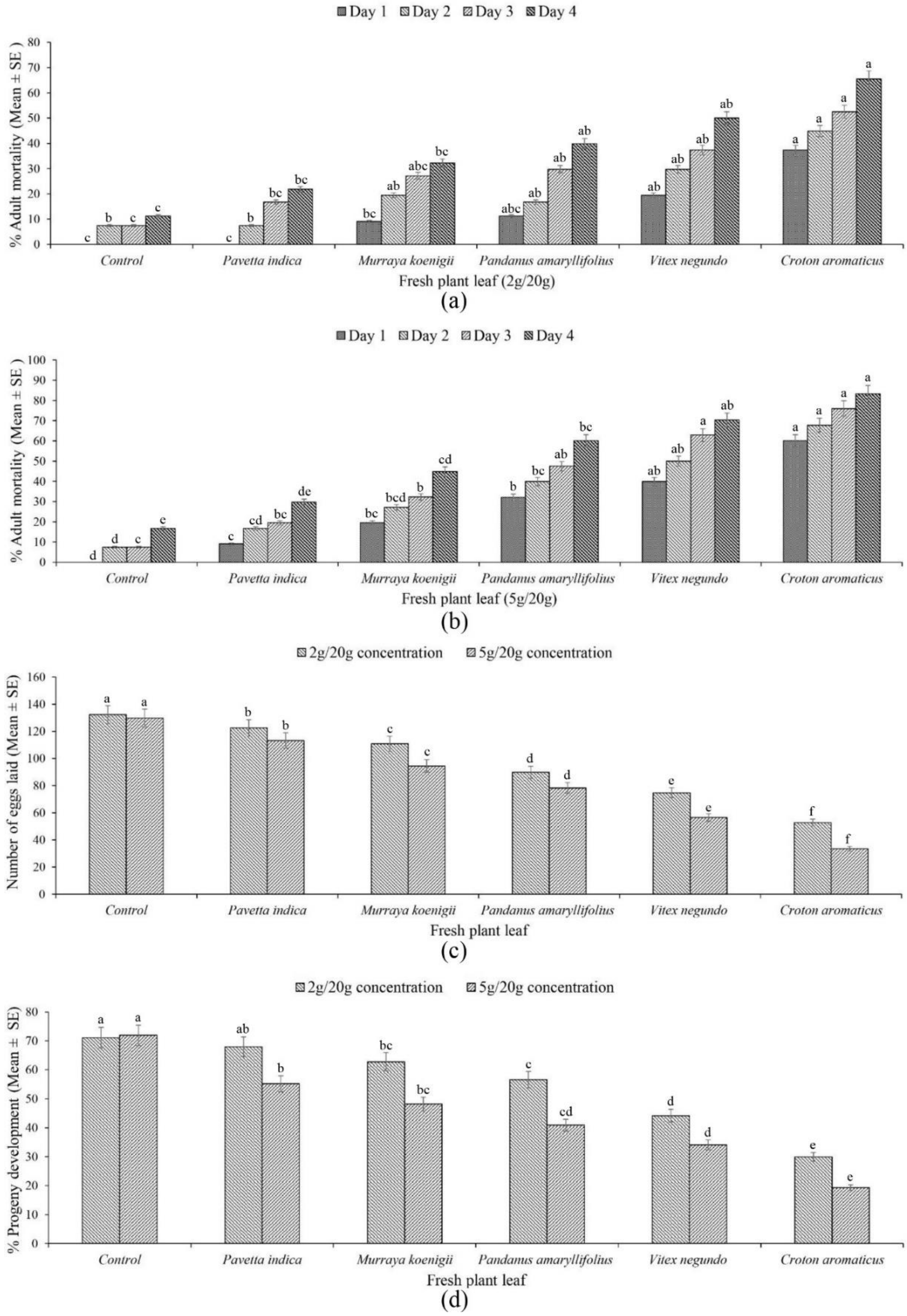

Figure 1: (a) % Adult mortality (2g/20g) (b) % Adult mortality (5g/20g) (c) number of eggs laid (d) % progeny development of *Callosobruchus maculatus* when treated with different fresh plant leaves: *Murraya koenigii*, *Pandanus amaryllifolius*, *Vitex negundo*, *Pavetta indica* and *Croton aromaticus*. Bars with the same letter are not significantly different (ANOVA, Tukey's test, $P < 0.05$).

treated with fresh plant leaves of *C. aromaticus* (Figures 1c and 1d). Moreover, the number of eggs laid and the percentage of progeny development declined with the increased concentration of fresh plant leaves. This indicates that the phytochemicals of *C. aromaticus* leaves in fresh form have a greater impact on the suppression of the number of eggs laid and progeny development of *C. maculatus* than the other plant leaves tested.

Moreover, the application of plant powders on cowpea seeds showed a significant effect on adult mortality, the number of eggs laid and progeny development. A significant difference of percentage adult mortality of *C. maculatus* at 2 g application of different plant leaf powders was observed after 1 day ($F_{5,18}$=41.06, P=0.00), 2 days ($F_{5,18}$=19.36, P=0.00), 3 days ($F_{5,18}$=20.81, P=0.00) and 4 days ($F_{5,18}$=17.64, P=0.00) of exposure to the treatments (Figure 2a). Similarly, percentage mortality of *C. maculatus* was significantly affected by the application of 5 g of different plant leaf powders after 1 day ($F_{5,18}$=44.91, P=0.00), 2 days ($F_{5,18}$=49.83, P=0.00), 3 days ($F_{5,18}$=34.98, P=0.00) and 4 days ($F_{5,18}$=24.05, P=0.00) of exposure to the treatments (Figure 2b). However, the maximum mortality was observed in seeds treated with both 2 g and 5 g of *C. aromaticus* whereas the minimum mortality was recorded in seeds treated with both 2 g and 5 g of *P. amaryllifolius* (Figures 2a and 2b). Furthermore, results revealed that an increase in exposure time to plant powders increases the mortality of adult *C. maculatus*. Ileke and Oni (2011) stated that mortality of adult *Sitophilus zeamais* increases with the increased time of exposure to plant powders such as *Azadirachta indica, Alstonia boonei, Garcina kola* and *Moringa oleifera.* According to the current study high concentrations of plant powders showed an increased effect on mortality irrespective of the type of plant leaf powder. Usually, levels of efficacy vary according to test concentration with the highest concentration tested providing the best control (Belmain *et al*., 2001). Similar results were demonstrated when *M. koenigii, P. amaryllifolius* and *P. indic*a plants were screened for repellent activity against *Sitophilus oryzae* (Gunarathna &

Karunaratne, 2009). Air drying of plant leaves may lead to changes in the active chemical components in different plant leaves. During the present study, it was observed that there is a choky odour present in dry plant powders. The odour varied among different leaf species and *C. aromaticus* had a strong choky odor than other plants. Ileke *et al*. (2013) stated that the high mortality caused by the powder of *Citrus sinensis* on *C. maculatus* might be due to a strong choky odour that disrupt the respiratory activity of the beetles.

A significant effect was observed in the mean number of eggs laid by *C. maculatus* at 2 g ($F_{5,18}$=842.94, P=0.00) and 5 g ($F_{5,18}$=878.43, P=0.00) application of different plant leaf powders (Figure 2c). Besides, the percentage progeny development showed a significant effect with different types of plant leaf powders at both 2 g ($F_{5,18}$=68.09, P=0.00) and 5 g ($F_{5,18}$=92.01, P=0.00) concentrations (Figure 2d). However, the maximum number of eggs laid, and the percentage progeny development was observed once the seeds were treated with both 2 g and 5 g concentrations of *P. amaryllifolius* powder. In contrast, the minimum was observed once the seeds were treated with a powder of *C. aromaticus*. Correspondingly, the number of eggs laid and the percentage of progeny development declined with the increase in the concentration of the plant powder. (Figures 2c and 2d). In contrast to the current study, early literature stated that *M. koenigii* is one of the most effective plants in reducing the oviposition of *Callosobruchus chinensis* at a dose of 2% (w/w). Accordingly, the F1 emergence from the infested chickpea decreased significantly with powders of *M. koenigii* (Shukla *et al*., 2007).

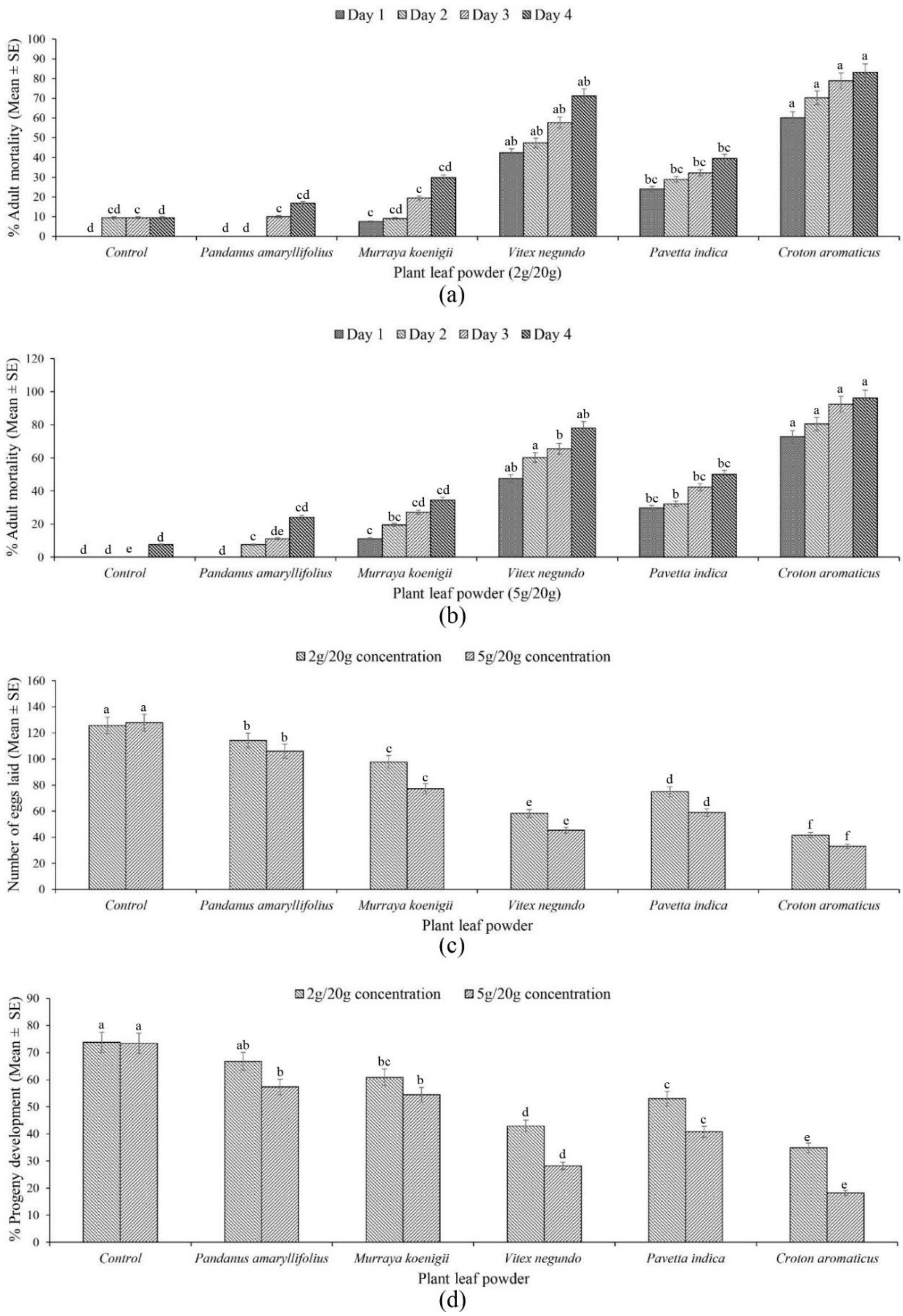

Figure 2: (a) % Adult mortality (2g/20g) (b) % Adult mortality (5g/20g) (c) number of eggs laid (d) % progeny development of *Callosobruchus maculatus* when treated with different plant leaf powders: *Murraya koenigii*, *Pandanus amaryllifolius*, *Vitex negundo*, *Pavetta indica* and *Croton aromaticus*. Bars with the same letter are not significantly different (ANOVA, Tukey's test, $P < 0.05$).

The lethal effect of the plant powders on the bruchid could be because of contact toxicity. Insects breathe through a tracheal system that usually open at the surface of the body through spiracles (Adedire *et al*., 2011). The spiracles might have been blocked by the plant powders thereby leading to the suffocation of the adult bruchid. The plant powders also prevented oviposition, adult emergence, reduction in weight loss and damage to the seeds by *C. maculatus* on treated cowpea seeds. Furthermore, these powders inhibit locomotion which affects mating activities and sexual communication (Okosun & Adedire, 2010; Ileke & Oni, 2011).

Musa *et al*. (2009) described the efficacy of mixed leaf powders of *Vernonia amygdalina* and *Ocimum gratissimum* against *C. maculatus*. Later, Akunne *et al*. (2014) testified the efficacy of mixed application of *V. amygdalina* and *A. indica* leaf powders against adult *C. maculatus*. They confirmed that mixing plant powders increases mortality and lowers the adult emergence of *C. maculatus*. Thus, in the current study, plant leaf powders were combined to detect the most effective combination for the control of *C. maculatus*. A significant difference in adult mortality was observed after 1 day ($F_{4,15}$=30.98, P=0.00), 2 days ($F_{4,15}$=53.23, P=0.00), 3 days ($F_{4,15}$=28.75, P=0.00) and 4 days ($F_{4,15}$=45.50, P=0.00) of application of different combinations of plant leaf powders (Figure 3a). Accordingly, the maximum mortality of *C. maculatus* was recorded from the mixture of *C. aromaticus – P. amaryllifolius – V. negundo* (WPN) combination from all the days of exposure. However, a 100% mortality was observed from the same combination after 4 days of exposure. This may be because of the increased contact toxicity of *C. aromaticus* from the chemical compounds produced from *V. negando* and *P. indica* plant powders. The minimum percentage mortality (19.48%) was recorded from the *P. amaryllifolius – M. koenigii* (RK) plant leaf combination on all days of exposure (Figure 3a). But the percentage mortality of *C. maculatus* would be higher when *P. amaryllifolius* (24.03%) and *M. koenigii* (34.56%)

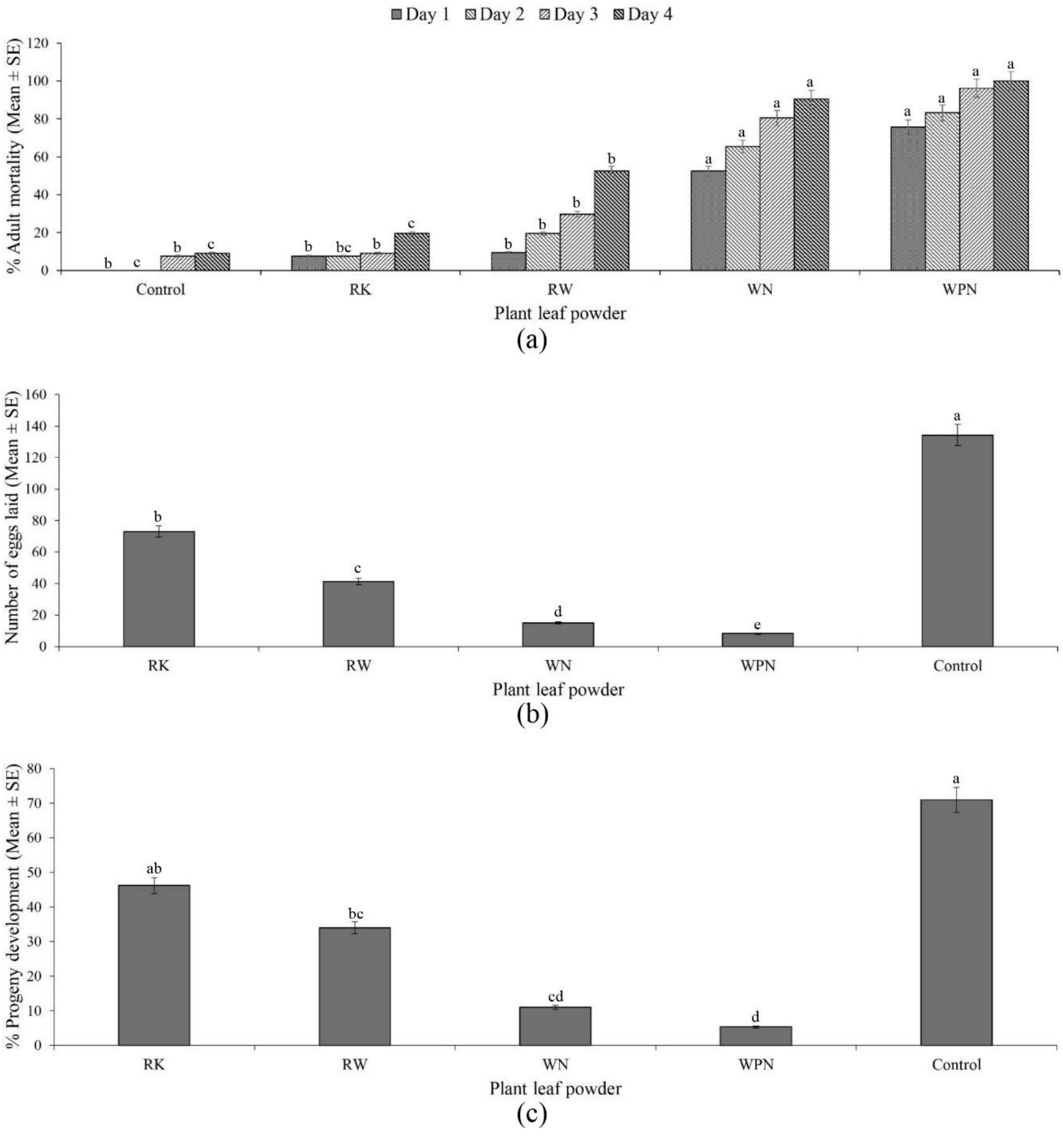

Figure 3: (a) % Adult mortality (b) number of eggs laid (c) % progeny development of *Callosobruchus maculatus* when treated with combinations of plant leaf powders: *Pandanus amaryllifolius* - *Murraya koenigii* (RK), *Pandanus amaryllifolius* - *Croton aromaticus* (RW), *Croton aromaticus* - *Vitex negundo* (WN), *Croton aromaticus* - *Pandanus amaryllifolius* - *Vitex negundo* (WPN). Bars with the same letter are not significantly

different (ANOVA, Tukey's test, P< 0.05) powders are applied alone. The reduction of toxicity may be due to the antagonistic effect caused by the mixing of these plant powders. However, *P. amaryllifollius* reduces the toxicity of *C. aromaticus* and *C. aromaticus* increases the toxicity of *P. amaryllifollius*. Therefore, the use of *C. aromaticus* alone gives better results than mixing with *P. amaryllifollius.*

Furthermore, the number of eggs laid ($F_{4, 15}$=2195.93, P=0.00; Figure 3b) and the percentage of progeny development ($F_{4, 15}$=20.16, P=0.00; Figure 3c) of *C. maculatus* were significantly affected by different combinations of plant powders. The number of eggs laid, and the percentage of progeny development were lowest once the cowpea seeds were treated with the combination of *C. aromaticus – P. amaryllifolius – V. negundo* (WPN) plant leaf powder. This may be due to the synergistic effect of *V. negando* and *P. indica* on *C. aromaticus*. However, the number of eggs laid, and the percentage of progeny development were highest once the seeds were treated with the combination of *P. amaryllifolius – M. koenigii* (RK) plant leaf powder (Figure 3b and 3c). Although the values are high when compared with the other combinations of plant powders, the values are a little lower compared to the effect of single leaf powders of *P. amaryllifolius* and *M. koenigii.* Thus, this shows that the combined effect has some potential to increase the toxic effect of a single leaf powder on the development of *C. maculatus.*

CONCLUSIONS

The current study indicates that treating cowpea seeds with fresh plant leaves and plant leaf powder of C. *aromaticus* successfully results in the highest adult mortality, lowest oviposition and progeny development in cowpea bruchid. Thus, it is eligible to consider the application of fresh plant leaves or plant leaf powder of *C. aromaticus* to control the cowpea bruchid infesting cowpea seeds in storage.

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