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Efficacy of botanicals in controlling F_1 progeny production in *Tribolium castaneum* (Herbst)

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Abstract
Laboratory bioassays (30±2 °C temperature, 70±5% r. h. and 12:12, L: D conditions) were performed to evaluate the insecticidal effects of eight different plant extracts against *Tribolium castaneum* (Herbst). Dichloromethane extracts of *Lantana camara*, *Calotropis procera*, *Polygonum hydropiper*, *Jatropha curcas*, *Datura metel*, *Annona squamosa*, *Vitex negundo* and *Murraya koenigii* were applied to whole-wheat flour at different doses of 40, 50, 60 and 75 mg respectively and placed in different jars. Hundred newly emerged adults were introduced into each jar for mating, oviposition and later removed from the corresponding jars. The emerging adults (F_1) were counted and weighed. Results were analyzed statistically. It was observed that all the plant extracts significantly reduced the production of F_1 individuals (F_1 progeny production) with *Calotropis procera* and *Jatropha curcas* showing the highest efficacy.

Keywords: Pest, botanicals, environment, control

1. Introduction
Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is a stored grain pest and is has a worldwide distribution. It is reddish brown in colour and is commonly known as the red flour beetle. It is a pest of cereals, flour, beans, pasta, pet food, dried flowers as well as museum specimens, chocolates, seeds and nuts (Via, 1999; Weston and Rattlingourd, 2000) [1, 2]. The egg to adult life cycle takes about 40-90 days and they may live to a period of three years (Rebecca and Thomas, 2003) [3]. Red flour beetles are prolific breeders. They continuously breed in warm areas round the year. The eggs, larvae, pupae, adults, their excreta and body parts contaminate the grains and make it unfit for consumption by man. They cause increase in moisture content and rise in temperature that leads to the production of molds and growth of toxigenic species (Magan *et al*., 2003) [4]. Post- harvest losses due to insect infestations and secondary attack by bacteria and fungi in storage conditions account for 10% and 20% of the total production of cereals and legumes in developed and developing countries, respectively (Mason and McDonough, 2012; Nayak and Daglish, 2018) [5, 6]. Though chemical pesticides are used to control their population; these have many harmful effects on human health, environment and on non-target organisms. They have after-effects like pest resurgence and pesticide resistance that becomes a major problem in pest management. There are several environmental and safety concerns specially on use of chemicals like methyl bromide and dichlorvos, which have been in use for insect pest eradication. Hence, there is a prime need for evaluation and development of new insecticidal formulations for the control of stored product insect pests (Daglish *et al*., 2018) [7].
Plants provide prospective alternatives to currently used toxic chemical insecticides. Plants contain bioactive compounds; these constitute a defence system in plants to protect them against attack of insects and other diseases. The bioactivities associated with the secondary metabolites present in plants counteract the activities of varied species of insects (Isman, 2006, 2008) [8, 9]. Thus, they can suffice as control measure against insect pest populations. Extracts derived from plants are locally and easily available, less expensive, biodegradable, have diverse modes of action, and have low toxicity to non-target organisms. They can be incorporated into integrated pest management systems and help in contributing to sustainable agricultural production (Geraldin *et al*., 2020) [10].
Toxicity was observed in insect pests of storage like *T. castaneum* (Herbst), *Oryzaephilus surinamensis* (L.), *Sitophilus oryzae* as well as *Rhyzopertha dominica* upon application of the essential oil of spices like anise (*Pimpinella anisum* L.) and peppermint (*Mentha piperita* L.)

(Shaaya *et al*., 1991) [11]. Also, the essential oil of garlic (*Allium sativum*) showed insecticidal properties upon application on *T. castaneum* and *Sitophilus zeamais* (Motschulsky) (Ho *et al*., 1996) [12]. Similarly, essential oil extracted from seeds of nutmeg (*Myristica fragrans* Houtt) (Huang *et al*., 1997) [13] and bark of cinnamon (*Cinnamomum aromaticum* Nees) (Huang and Ho, 1998) [14] exhibited toxicity against *S. zeamais* and *T. castaneum.* Extracts of *L. camara* reduced the emergence of adults, induced delay in development and produced lower fecundity in *R. dominica* (Singh *et al*., 1996) [15]. Leaf extracts of *J. curcas*, *L. camara*, *C. procera* and *D. metel* exhibited toxicity against *T. castaneum* (Kalita and Bhola, 2014) [16]. Twenty-three different plant extracts were evaluated for their efficacy on *Callosobruchus maculatus* (Boeke *et al*., 2004) [17]. Promising results were found when pea flour extract was enriched with protein and used against *S. oryzae* to protect rice in storage (Kumar *et al*., 2004) [18].

The present study was taken up to observe how the eight plant extracts affected the F_1 progeny production in the tested insect *T. castaneum*.

2. Materials and Methods

2.1 Insect rearing and preparation of plant extracts

T. castaneum was cultured in the laboratory at ambient conditions of temperature, relative humidity and photoperiod of (30±2°C), (70±5%) and (12:12, L:D conditions) respectively. Leaves of *Lantana camara*, *Calotropis procera*, *Polygonum hydropiper*, *Jatropha curcas*, *Datura metel*, *Annona squamosa*, *Vitex negundo* and *Murraya koenigii* were collected, washed and air-dried for three to five days, followed by drying in the oven at 40°C for 24 hours after which they were powdered in an electric blender. The extraction was carried out in Soxhlet apparatus using dichloromethane. The extract was collected, air-dried to vaporize the solvent and covered by aluminum foil. This was kept at 4°C and stored for conduction of the bioassays.

2.2 Application of the extracts

100g whole-wheat flour was sterilized in an oven for 12h at 30°C. Thereafter, 40, 50, 60 and 75 mg of the extracts of the tested plants were added to the sterilized flour for each bio-assay respectively. The flour was then shifted to jars measuring 500 ml. In each jar, hundred newly emerged adults were placed; then kept in an incubator for mating and oviposition at a temperature of 30°C for 4 days. The flour was then sieved to remove the adults and placed in different jars as per the treatments applied. The mouths of the jars were tied with muslin cloth and kept at a temperature of 30°C for emergence of adults. The F_1 adults were counted and weighed after complete emergence. Five replicates were taken for each treatment and one control was set up. Results were statistically analyzed.

3. Results

Extracts from eight plants were applied to the parent insect *Tribolium castaneum* in order to evaluate their effect(s) on F_1 generation. It was observed that in all the concentrations, the tested plants exhibited significant reduction in the number of adults of F_1 generation. The bioassay using dichloromethane extract of the eight plants on production of F_1 generation is presented in Table 1. *C. procera* (42.7-58.8) exhibited highest effectiveness with the production of least number of progeny followed by *J. curcas* (41.4-66.6), *P. hydropiper* (54.3-56.1), *D. metel* (43.2-69.4), *V. negundo* (55.5-62.4), *L. camara* (55.2-68.4), *A. squamosa* (57.9-65.2) and *M. koenigii* (61.9-70). As the concentration increased from 40mg to 75mg, there was decrease in the number of F_1 adults' emergence. The highest number of F_1 progeny was in the control bioassay with a production of about 95.5 individuals, which was much higher than any of the treated bioassays.

The weight of the F_1 progeny with regard to a single insect did not vary significantly as compared to the control in all the bioassays.

3.1. Effect of the extracts at a concentration of 40 mg on F_1 progeny

For all the plant extracts tested, the numbers of F_1 progeny produced were less than that of the control. *P. hydropiper* showed the highest efficacy with an average emergence of 56.1 individuals against control, which showed an emergence of 95.4 number of individuals of *T. castaneum.* The order of efficacy was *P. hydropiper> C. procera> V. negundo> A. squamosa> J. curcas> L. camara> D. metel> M. koenigii* with an emergence of 56.1, 58.8, 62.4, 65.2, 66.6, 68.4, 69.4 and 70 numbers of *T. castaneum* individuals respectively. Thus, all the tested extracts could reduce the production of F_1 individuals significantly. However, there was no significant reduction in weight of the emerged individuals of F_1 progeny and was almost same as that of control.

3.2. Effect of the extracts at a concentration of 50 mg on F_1 progeny

C. procera at a concentration of 50mg showed the highest efficacy in controlling F_1 progeny production. It lead to the emergence of 54.4 number of individuals as compared to 95.4 in control. The order of efficacy was *P. hydropiper>J. curcas>V. negundo>D. metel>A. squamosa>L. camara>M. koenigii* with 55.8, 58.4, 59.9, 61.6, 63.2, 65.4, 68.7 average numbers of emerging individuals respectively. There was no significant reduction in weight of the emerged individuals of the F1 progeny. But in the treatment with *L. camara*, the weight was found to be 1.7mg against 2.25 in control; however, no significant differences in weight could be observed.

3.3. Effect of the extracts at a concentration of 60 mg on F_1 progeny

J. curcas showed the highest efficacy in controlling the emergence of F1 individuals at 60mg concentration. Average weight of emerged individual was 1.58mg against 2.25mg in control. However, it was not significantly different from control. The order of efficacy was *J. curcas>C. procera>D. metel>P. hydropiper>V. negundo>L. camara>A. squamosa>M. koenigii* with emergence of an average of 42.2, 45.0, 46.2, 54.0, 57.0, 57.4, 61.8, 65.8 number of individuals in F_1 progeny against 95.4 numbers in control.

3.4. Effect of the extracts at a concentration of 75 mg on F_1 progeny

The lowest emergence of F_1 individuals was in the bioassay treated with 75mg extract of *J. curcas.* The order of efficacy was observed to be *J. curcas>C. procera>D. metel>L. camara>P. hydropiper>V. negundo>A. squamosa>M. koenigii* with emergence numbers of an average of 41.4, 42.7, 43.2, 54.3, 55.2, 55.5, 57.9, 61.9 individuals which is significantly different from control where the observed number were 95.5 individuals. Though there was no

significant difference in weight, however, the bioassays treated with *J. curcas* and *L. camara* resulted in production of individuals weighing 1.56mg and 1.66mg respectively against the control, which was 2.25mg.

Tables and Figures

Table 1: Effect of dichloromethane (DCM) plant extract on F_1 progeny

Plant	Concentration							
	40 mg/100g wheat Flour		50 mg//100g wheat Flour		60 mg//100g wheat Flour		75 mg/100g wheat Flour	
	No. of F1 adults	Wt. of F1 progeny (mg)	No. of F1 adults	Wt. of F1 progeny (mg)	No. of F1 adults	Wt. of F1 progeny (mg)	No. of F1 adults	Wt. of F1 progeny (mg)
Control	95.4±3.65	215.6 (2.25)	95.4±3.6	215.6 (2.25)	95.4±3.6	215.6 (2.25)	95.4±3.6	215.6 (2.25)
C. procera	58.8±5.4*	130.4±5.2 (2.21)	54.4±2.8*	106.6±7.1 (1.96)	45.0±5.2*	83.2±9.9 (1.80)	42.7±5.4*	79.3±8.3 (1.86)
J. curcas	66.6±4.22*	138.2±10.4(2.07)	58.4±7.1*	111.4±14.1(1.91)	42.2±7.1*	66.8±11.2(1.58)	41.4±4.8*	64.4±13.1(1.56)
P. hydropiper	56.1+5.1*	128.4±8.9(2.29)	55.8±5.3*	126.80±9.9(2.27)	54.0±5.4*	115.1±6.9(2.14)	54.3±7.0*	118.7±7.3(2.18)
D. metel	69.4±4.39*	124.2±7.7(1.78)	61.6±5.1*	123.2±16.3(2.0)	46.2±6.3*	86.8±12.5(1.88)	43.2±3.7*	83.8±9.4(1.93)
V. negundo	62.4±4.1*	126.0±10.1(2.1)	59.9±3.2*	124.4±12.1(2.08)	57.0±4.8*	119.4±11.8(2.09)	55.5±4.9*	114.0±6.2(2.05)
L. camara	68.4±5.94*	123.2±14.2(1.80)	65.4±5.4*	111. 0±9.3(1.7)	57.4±9.9*	93.0±16.5(1.62)	55.2±6.1*	91.5±12.5(1.66)
A. squamosa	65.2±3.7*	136.6±12.3(2.09)	63.2±4.8*	134.0±12.3(2.12)	61.8±6.1*	130.0±15.4(2.10)	57.9±5.6*	125.7±5.7(2.17)
M. koenigii	70.0±6.20	140.2±7.8(2.0)	68.7±6.7*	135.2±8.2(1.97)	65.8±3.5*	124.6±7.9(1.89)	61.9±3.6*	118.1±9.1(1.90)

(Figure in parenthesis represent the average weight of *T. castaneum*.
Average weight of single insect (of F_1 progeny) at varied concentrations of same/ different plant extract do not differ significantly
*Significantly lower than control)

4. Discussion

The results showed that the *T. castaneum* population level were lower in the treated wheat samples as compared to the ones in the control. Similar results were observed on several insect pests using varied plant extracts. Extracts of *Peganum harmala, Ajuga iva* and *Aristolochia baetica* inhibited F1 progeny production in *T. castaneum* (Jbilou *et al*., 2008) [19]. *P. harmala* seeds contain bioactive compounds that cause developmental, physiological and histopathological effects in *T. castaneum.* Methanol extracts of *Peganum harmala* (L.) seeds when mixed with food led to inhibition of larval growth, reduced food intake, increased duration of larval period and inhibition of F1 progeny production in *T. castaneum* (Jbilou and Sayah, 2008) [20]. Extracts from plants and their oils contain camphor, eugenol, 1, 8-cineole which have properties that can result in inhibiting production of progeny and causing mortality in insect pests (Obeng-Ofori and Reichmuth, 1997) [21]. Inhibition of adult emergence was also reported in *Sitophilus granaries* and *Rhyzopertha dominica* treated with gounded seeds of black pepper (*Piper nigrum* L.) and fruit powder of red pepper *(Capsicum annum* L.) (Shabnam and Nouraddin, 2010; Singh *et al*., 2009) [22, 23]. *Calotropis procera* significantly reduced the number of adult emergence of *R. dominica* Fabr. (Jacob and Sheila, 1993) [24].

All the eight plant extracts exhibited significant reduction in the number of F_1 adults of *T. castaneum* at the different tested concentrations. *C. procera* was the most effective, which resulted in the production of least number of F_1 progeny. The order of efficacy was *C. procera*> *J. curcas*> *P. hydropiper*> *D.metel*> *V. negundo*> *L. camara*> *A. squamosa*> *M. koenigii*. The adult emergence of *C. procera* was in the range of 58.8, 54.4, 45 and 42.7 against 40, 50, 60 and 75 mg/ 100 gram flour respectively. As the concentration increased from 40mg to 75mg, the emergence of F_1 individuals reduced in number. A similar study revealed the potency of *Piper nigrum* powder on *Callosobruchus maculates.* It reduced oviposition and adult emergence in a significant manner (Rajapakes, 1990) [25]. The two varieties of pepper (black pepper and red pepper) helped to reduce the emergence of adults of *Callosobruchus chinensis* L. (Morallo – Rejesus *et al*., 1990) [26]. Both larvae and adult development of *T. castaneum* in grains of millet was reduced by using clove and West African black pepper (Lale and Ajayi, 2000) [27]. Edible fatty oils of *Moringa oleifera, Sesamum indicum* and *Olea europaea* stopped progeny emergence of *T. castaneum* in broken millet (Appert, 1987) [28]. F_1 progeny production in *T. castaneum* was totally inhibited by nutmeg oil (Huang *et al*., 1997) [13] and by *Elettaria cardamomum* (L.) oil (Huang *et al*., 2000) [29] when applied to the diet. Azadirachtin significantly reduced offspring production in *T. confusum* (Athanassiou *et al*., 2005) [30]. There was observation of F_1 inhibition in *Zabrotes subfasciatus* upon application of *Chenopodium ambrosioides, A. indica* and *Datura stramonium* in powder form (Araya and Emana, 2009) [31].

The present study showed that there was no significant difference in the weight of single insect of the F_1 generation at diverse concentrations of the extracts of the same as well as the different plants tested. However, in the bioassays using *J. curcas* and *L. camara* extracts, there was observance of a slight decrease in the weight of emerging individuals. Nicotine application in *Heliothis virescens* produced lesser number of survivors, decrease in weight of pupae and longer developmental period (Gunasena *et al*., 1990) [32]. F_1 progeny production was inhibited in *C. maculatus*, by applying *Gnidia kraussiana* Meisn extracts (Kosini and Nukenine, 2017) [33]. *Hemizygia welwitschii* extracts were toxic against adults of *C. maculatus* and significantly inhibited emergence of the F_1 progeny in treated grains (Tagne *et al*., 2019) [34].

5. Conclusion

The extracts of the tested plants exhibited positive results in reducing the number of F_1 individuals significantly. *C. procera* and *J. curcas* showed highest efficacy producing lesser number of F_1 progeny followed by *P. hydropiper*. The extracts of *D. metel* was more effective than *V. negundo*, while *L. camara* showed more potency than *A. squamosa* and *M. koenigii.* However, all the plant extracts showed good efficacy as compared to the control thus revealing the possibility of their use for controlling the population of *T. castaneum.* The isolation and identification of the active ingredients from botanicals and the mode of action on insect pests can be used for combating pests of food commodities,

specifically in storage. These botanicals are eco-friendly, locally available and cheaper than their chemical counter-parts. They pose low risk hazards and have less residual toxicity on non-target organisms. Their isolation and use to combat insect pests can usher in an era of health and environment friendly insecticides.

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