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RESEARCH ARTICLE

ASSESSING THE USE OF *CROTON TIGLIUM* L.OIL AS A SEED-PROTECTANT AGAINST *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA:CHRYSOMELIDAE)

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ABSTRACT

A study was carried out to protect *Vigna radiata* L. seeds from the common storage product pest, the pulse beetle, *Callosobruchus maculatus* (F.) by using oil prepared from the seeds of a wild plant, *Croton tiglium* (L.). This oil was found to be toxic to *C. maculatus* adults. The 24 and 96 h LC_{50} values (in ml/100g grain) were 0.93 and 0.74 respectively. *C. tiglium* oil was found to have an effective ovicidal action. At oil concentrations of 0.6 and 0.8 ml/100g grain, adult emergence was as low as 26 and 9 percent respectively, compared to 98 percent emergence in the control group. The grains drenched in *C. tiglium* oil germinated normally, indicating the non-toxic nature of the oil on the grain endosperm, and hence, the oil is very effective in protecting the grains without affecting seed germination. At the highest drenching concentration of 1.0 ml/100g grain, the mean germination percentage of *V. radiata* seeds was 96, whereas 100 percent germination was recorded in the control group. The oil concentration at which high ovicidal activity was recorded, failed to affect the germination potential of the grains.

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INTRODUCTION

Seeds are future generative materials and the seed quality reflects on the yield of any plant material. Further these seeds are highly proteinaceous food commodities, preferred for human consumption. Insects damage seeds in storage, affecting their marketability for domestic use and germination potential when used as seed material. During storage, the seeds have to be protected from insects, till they can be sowed or put to other uses. Insect invasion causes reduction in weight, market value and germination of seeds as surveyed by the International Institute of Tropical Agriculture (IITA, 1989).Successful seed storage is the key to farmers' seed security and may also enable communities to generate income through collecting, storing and selling seeds (Wambugu *et al.*, 2009).Among the important insect pests of stored seeds, the cowpea beetle, *C. maculatus* is highly significant (Helaly, 2018), as it causes quantitative and qualitative loss of very important pulses like *Vigna unguiculata* (L.) Walp. and *Vigna radiata* (L.) R. Wilczek, resulting in seed perforation and reduction in weight, leading to decreased market value and scaling down of seed germination potential (Shukla *et al.,* 2007). Detailed inventory of the enormous environmental and health problems caused due to the toxic residues left behind by chemical pesticides, used in the management of stored grain pests, make grain storage experts plan for alternate methods of pest control (Owolabi *et al.,* 2009) and one among these is the use of botanical pesticides which is very promising because of several distinct advantages (Parugrag and Roxas, 2008). As a defence mechanism against insects, the plants develop secondary metabolites which are excellent sources of insecticidal substances (Koul *et al*., 2008 and Saeidi and Pezhman, 2018). These metabolites are obtained in the form of powders and oils with the ability to control various insect pests. Traditional use of plant products in the management of stored grain pests has also been streamlined for better efficacy. For a long time, neem seed powder (*Azadiracta indica* Juss.), pepper fruit seed powder (*Dennittia tripetala* Bak. f.)and black pepper (*Piper guinensis* Schum and Thonn.) havebeen used for protecting maize and cowpea seeds from storage pests, that too without any adverse effects on the viability of the seeds (Duruigbo, 2010).

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Much similar to plant powders, oils extracted from some plants are highly toxic to insect pests of seeds. These oils may be applied on the surface of the seeds for inhibiting seed pests (Oparaeke *et al.,* 2005). Neem oil and moringa seed oil (*Moringa oleifera*) were found to be effective in the preservation of cowpea, *V. unguiculata* grains against the cow pea bruchid, *C. maculatus* (Ilesanmi and Gungula, 2010). Udo (2011) evaluated the effect of palm oil and groundnut oil against cow pea weevil *C. maculatus* on stored cow pea, *V. unguiculata*. Neem, Castor, Eucalyptus, and Sunflower oils have been used for protecting pigeon pea against damage by *C. maculatus* (Lal and Deepshika, 2012). The possibility of using the seeds of wild plants for extracting insecticidal oils is explored intensely. Oil, extracted from the seeds of one such wild plant, *Croton tiglium* L. (Euphorbiaceae) a shrub native to South East Asia is used in many insecticidal formulations taking advantage of its biocidal property (Jangid and Jadhav, 2018). Dhoutia *et al*. (2015) showed that the root of *C. tiglium* had remarkable mosquito larvicidal activity. In the present study, the possibility of using *C. tiglium* oil as a seed-protectant against the cowpea seed beetle, *Callosobruchus maculatus* Fab. was assessed based on the following preliminary investigations.

- The toxicity of *C. tiglium* oilto *C.maculatus*
- Developmental success of *C.maculatus* eggs laid on *V.radiata* smeared with low
- concentrations of *C.tiglium* oil
- Studying the germination efficiency of *V. radiata* seeds treated with *C. tiglium* oil

MATERIALS AND METHODS

Insect cultures: Cultures of *C. maculatus* were maintained in the Zoology Research Laboratory of Scott Christian College (Autonomous), Nagercoil. The stock insects were obtained from a private warehouse in Nagercoil Town (8° 10' 28.0200" N and 77° 25' 55.7724" E), Kanyakumari District. The insects were maintained in large plastic containers on whole green gram grains (*V. radiata*).

Procurement of seeds and extraction of oil: *C. tiglium* seeds obtained from the local native Ayurvedic medical shop, were sundried and ground in an electric blender and the ground material was stored in large glass containers. Exactly 50 g of ground *C. tiglium* seed powder were weighed in a sensitive electronic balance, wrapped in white muslin cloth and fastened with a twine. The packed material was placed in a 1000 ml Soxhlet extraction apparatus and the extraction was made at 40-60^{o}C with acetonitrile solvent. After extraction of the oil, the solvent was recovered using a rotary vacuum evaporator.

Toxicity Bioassay: Samples consisting of exactly 100 g of *V. radiata* seeds were placed inside 250 ml transparent plastic containers. *C. tiglium* oil was applied on the surface of the grains at different concentrations, ranging from 0.2 to 1.0 ml/100g of grain. The containers with the grains were agitated well in an electrical shaker to ensure a uniform application of the oil on the seed coat. For each concentration, about six replicates were maintained. In each container, 20 adult *C. maculatus* were released and the containers were kept closed. The mortality of *C. maculatus* in each container was observed for 5 days and mortality of the beetles beyond this time limit was ignored, since the adults are non-feeding and normally die in about 7-8 days after fulfilling their reproductive functions. Control seeds were kept without any oil treatment. Probit analysis was carried out to analyze the response of *C. maculates* to *C.tiglium* oil and n hr LC_{50} values were calculated (Finney, 1971).

Ovicidal action and adult emergence: Exactly 100g of *V. radiata* seeds drenched with *C. tiglium* oil at 0.2, 0.4, 0.6, 0.8 and 1.0 ml/100g of grain were taken in transparent virgin plastic containers and about 5 mating pairs of *C. maculatus* were introduced into each of the containers. Random egg laying was allowed in each container and, grains with single eggs were removed. Sufficient number of containers was maintained in each concentration so that grains with single eggs could be collected in about 24 h. This was done to facilitate free oviposition and prevent overcrowding of eggs. Grains carrying single eggs were collected so that in each oil concentration about 150 eggs were available for incubation in a single day itself. The seeds with eggs, treated at different oil concentrations were checked for signs of emergence of adult *C. maculatus* after a period of 28 days. Observations were made at intervals of 2 days after 28 days and continued up to 36 days. Adult emergence was counter-checked by enumerating the number of perforated seeds. Early adult emergence was first recorded in the control groups, where the seeds were not drenched with *C. tiglium* oil. Counting was continued till the 36thday after which emergence of adults was not recorded. The total number of adults emerging from the incubation of 150 eggs was recorded for each concentration and percent emergence was calculated.

Seed germination test: Exactly 100g of seeds were separately drenched with oil at concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 ml/100g seeds, in different containers. For seed germination test, 10 seeds were randomly selected from each of the five different oil treatment groups. Separate control sets were also maintained. The seeds were sown in the soil, taken in small earthen pots and were watered regularly. Number of germinated seedlings was counted and percentage of seed germination was calculated. Six replicates were maintained.

RESULTS AND DISCUSSION

It is clear that *C. tiglium* oil is highly toxic to *C. maculatus* with a 96 h LC_{50} of 0.73 ml / 100 g of *V. radiata* grains. The quantity of oil [0.73 ml] may not be sufficient to freely drench all the grains, still significant mortality is recorded in *C. maculatus* moving around the grains (Table 1). The beetles browse around the grains and the females lay eggs on the grains. The oil applied on the grains is found to be toxic to them. Hence this oil may be used in ultra-low concentrations to deter as well as kill the cowpea beetles. Different oils exhibit toxic effects on *C. maculatus* at different doses. Ilesanmi and Gungula (2010) reported that cow pea samples treated with moringa oil at 0.5 to 1.0 ml/200g, failed to arrest *C. maculatus* population fully,leaving behind as much as 25 and 105 insects respectively, after 180 days of storage. At the same time, when the grains were treated with neem oil and a mixture of these two oils at 1.5 ml/200g concentration, weevil population growth was completely arrested indicating their potency and persistent retardant effect. Udo (2011) used palm oil and groundnut oil to control *C. maculatus* and recorded mortality levels of 20 and 51 percent respectively, after 96 h in the concentration of 10ml/40gm of grains. The LC50 values of Jojoba oil against *C. maculatus* were 12.019, 4.69, and 1.482

Table 1. Toxicity of *C. tiglium* oil to adult *C. maculatus*

Sl. No.	Period of exposure (h)	Lower Confidence Limit (LCL)	LC_{50} (in ml/100g grain-)	Upper Confidence Limit (UCL)
1	24	0.90	0.94	0.98
2	48	0.85	0.88	0.91
3	72	0.78	0.82	0.85
4	96	0.69	0.73	0.77

Table 2. Adult emergence from eggs deposited by 5 pairs of *C. maculates* on *V.radiata* grains treated with *C.tiglium* oil (n=6; $\bar{X}$±SD)

Sl. No.	Days after oviposition	Grain drenching concentration (ml oil / 100g grain)					
		Control	0.2	0.4	0.6	0.8	1.0
		No. of adults emerged					
1	28	4.2 ± 0.44	-	-	-	-	-
2	30	48.4 ± 0.54	12 ± 1.5 (-4.03)	7.5 ± 0.9 (-6.5)	-	-	-
3	32	66.8 ± 0.44	22.8 ±2.4 (-2.92)	13.8 ± 1.4 (-4.8)	4.8 ± 0.51 (-13.9)	-	-
4	34	22 ± 1	47 ± 4.1 (2.1)	25.8 ± 2.8 (1.2)	13.6 ± 1.7 (-1.6)	5.9 ± 0.49 (-3.7)	-
5	36	4.8 ± 0.44	60.8 ± 0.44 (12.7)	32.3 ± 4.1 (6.7)	19.8 ± 1.8 (4.1)	8.4 ± 0.94 (1.75)	-
Percent adult emergence		98	96	54	26	9	0

Note: 'deviation factor' within parentheses; t-test-all deviations significant at P≤0.05

Table 3. Germination potential of *V. radiata* seeds, 30 days after treatment with *C. tiglium* oil (n=6; $\bar{X}$ ±SD)

Sl. No.	Days of germination	Grain drenching concentration (ml oil / 100g grain)					
		Control0.20.60.1 0.140.18	0.20.20.60.1 0.140.18	0.40.20.60.1 0.140.18	0.60.20.60.1 0.140.18	0.80.20.60.1 0.140.18	1.00.20.60.1 0.140.18
		Percent Germination					
1	1	-	-	-	-	-	-
2	2	80.6 ± 0.54	79 ± 0.70 (-0.01)	76 ± 0.70 (-0.06)	76 ± 0.70 (-0.06)	74 ± 0.70 (-0.08)	74.8 ± 0.44 (-0.07)
3	3	95.4 ± 0.54	93.6 ± 0.54 (-0.02)	91.8 ± 0.44 (-0.04)	90.2 ± 0.44 (-0.05)	88.2 ± 0.83 (-0.08)	87.6 ± 0.54 (-0.08)
4	4	99.2± 8.1	98.8 ± 0.44 (-0.004)	97.8 ± 0.83 (-0.01)	96.4 ± 0.54 (-0.03)	94.8 ± 0.44 (-0.04)	93.8 ± 0.44 (-0.05)
5	5	100	100	99.8 ± 0.44 (-0.002)	98.8 ± 0.44 (-0.01)	97.4 ± 0.54 (-0.03)	95.6 ± 0.54 (-0.04)

Note: : 'deviation factor' within parentheses; t-test-deviations not significant at P≤0.05

at 24, 48 and 72 h respectively (Helaly, 2018). Saeidi and Pezhman (2018) showed that the 24 h LC_{50} (in µl/l air) values of *Mentha piperita* L., *Mentha pulegium* L., *Zataria multiflora* Boiss. and *Thymus daenensis* Celak plant oils to *C.maculatus* were 13.7, 95.80, 99.94 and 65.55. The different LC_{50} values mainly depend on the dosage of treatment and the response of the insect pests in question (Saljoqi *et al.,* 2006). The toxicity of the plant oils is due to the phytochemical content of the oils. Abulude *et al*. (2007) stated that the plant oils could also kill the insects by physical properties of coating on the body surface and blocking of respiration. Edwin and Jacob (2017) reported that the physicohemical contents of plants like *Allium sativum* L. (terpenoids), *Cordia milleni* Baker (oleinic, betulic, terpenoid quinones and triterpene derivatives), *Monidora myristica* (Gaertn.) (myristicine, p-mycene and terpene) *Xylopia aethiopica* (Dunal) and *Zingiber officinale* Roscoe (alpha zingiberine) were highly toxic to *C. maculatus*adults.The GCMS analysis of *C. tiglium* extract made by Jeya (2012) revealed the presence of Benzoic acid, Ricinoleic acid, Benzene dicarboxylic acid, 2,6-Adamantanedione, Tetradecanoic acid and hexadecadienoicacid. The same compounds were reported by Saputera *et al*.(2006) in their work. Hassan *et al*. (2010) identified Hexanedioic acid and 1, 2-Benzenedicarboxylic acid synthetic analogues act as acaricides which are used in orchards and also as inert ingredients in pesticides. Benzenedicarboxylic acid also acts as an insect repellent (Hassan *et al.*, 2010). The control eggs started eclosing on the 28th day and adult emergence was followed till the 36th day. This was done to ensure that all the embryos, within the grains, that showed positive development eclosed within the allowed period of observation. Eclosion was significantly lower in higher concentrations of 0.4, 0.6 and 0.8ml /100g grains. At 1ml concentration, no eclosion was observed even though at this concentration, the beetles oviposited on the grains. The overall eclosion was as low as 9 percent in 0.8 ml concentration. At 0.2 ml concentration, 96 percent eclosion was recorded (Table 2). The oil residues on the grain virtually affected the newly laid eggs, either preventing their development or aborting their embryonic stages. The grains are coated over with oil and the eggs were deposited on the grain surface that had a clear oil film. The eggs are covered over by an envelope on the outer side, but the grain surface is left free for the hatched –out larvae to enter into the grain. The oil is found in this egg-grain interface and a probable interaction between the oil and the egg envelope led to developmental failure. The young larval skins of the just hatched out larvae possibly were affected by the micro film of oil on the grain leading to future developmental anomalies.

The impact of different plant oils on oviposition and adult emergence of *C. maculatus* was investigated by various authors. Dauda *et al.* (2014) showed that mean number of eggs laid by adult *C. maculatus* on 10 g *Moringa oleifera* (Lam.) oil treated cow pea grains was 0.67±1.30 at 0.6ml concentration with no adult emergence. Lal and Deepshikha (2012) investigated the efficacy of eucalyptus oil, neem oil, sunflower oil and castor oil against the growth and development of *C. maculatus*. The minimum number of eggs laid on the seeds were 10.63, 14.52, 14.67 and 15.37 respectively and the percent adult emergence recorded at 1ml/Kg pigeon pea seeds were, 1.01 for eucalyptus oil, 1.15 for neem oil and 1.37for castor oil. *C. tiglium* oil was found to be highly effective in preventing adult emergence in *C. maculatus*. The results are in agreement with those of some investigators who tried to protect cow pea seeds from *C. maculatus*. Wahedi *et al*. (2013) showed that neem seed extract prevented emergence of F1 adults of *C. maculatus*. Ibrahim and Aliyu (2014) reported that the lowest mean number of holes was recorded in cow pea seeds treated with African nutmeg oil and moringa seed oil. Ganga and Dayanandan (2017) in their studies revealed that the number of eggs laid by *C. maculatus* decreased on treatment with plant extracts. Germination of *V. radiata* was tested in grain samples, separately subjected to five different oil concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 ml/100g grains.

These concentrations are the same as those tested for larval development. The additive values of grain germination were recorded till the 5th day. In the highest oil concentration of 1.0ml/100g grains, about 96 percent of the seeds germinated indicating that the germination potential of the seeds was not affected at a concentration, where egg development was totally absent (Table. 3). The oil coated on the surface did not affect the germplasm of the seed. Lal and Deepshikha (2012) in their study showed that the seed germination percentage of pigeon pea in neem oil, eucalyptus oil, castor oil and sunflower oil was not much affected when the seeds were treated with the oils at 1ml/Kg (84.72, 82.20, 81.01 and 81.09) and 3ml/Kg (82.96, 80.47, 78.23and 77.10 respectively).

It may be concluded that *C. tiglium* oil can be used as an ideal seed protectant because it kills stored grain pests like *C. maculatus*. It prevents embryonic development of the pest at low concentration and does not interfere with the germination efficiency of the treated grains. This oil would therefore bring down the population of the pest, retaining the germination potential of the seed. The result is further supported by Sathyaseelan *et al.* (2008) who reported that no significant negative effects were observed in the germination of green gram seeds after 0, 60 and 90 days of treatment with some indigenous pesticidal plant extracts. It may be assumed that the oil does not reduce the nutritional quality of the grain also. Udo (2011) reported that there was no significant difference in the percentage of germination of cow pea grains treated with ground nut oil and palm oil (87 and 80 percent respectively) compared to the control (100percent).In contrast to the present results, *Khaya senegalensis* (Desr.) A. Juss. seed oil which was studied by Bamaiyi *et al.* (2006), affected the viability of cow pea seeds. The insecticidal property of *C. tiglium* is not much analyzed. But the plant is used in the treatment of many diseases in Ayurvedha. The plant is found throughout India. Even though it is toxic, it is not lethal to human beings (Saranya *et al*., 2019). According to this study, *C. tiglium* oil may be used as a seed protectant as it has good insecticidal property.

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