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INFLUENCE OF SELECTED LEGUME SEEDS ON EMERGENCE OF *Callosobruchus maculatus* (F.) AND ITS SUSCEPTIBILITY TO *Azadirachta indica* (A. Juss) AQUEOUS LEAF EXTRACTS

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ABSTRACT

In the absence of effective protection, grains of legumes may be completely decimated when attacked in storage by the cowpea bruchid, *Callosobruchus maculatus*. The use of botanical insecticides has been reported as ecologically innocuous alternatives to conventional insecticides for the control of *C. maculatus* in stored legume grains. This study thus investigated how legume type influenced the emergence of *C. maculatus* and its susceptibility to the aqeous extract of neem leaf (a botanical based insecticide). Thirty (30) *C. maculatus* adults from the same population were reared for 4 generations on improved varieties of cowpea (IT89KD391), mung bean (NM 92) and soybean (TGX 1448) seeds under laboratory conditions. While both cowpea and mung bean supported the emergence of adult *C. maculatus* in all the four filial generations studied, emergence in soybean terminated after first filial generation. Susceptibility of fourth filial generation adult beetles from cowpea and mung bean to neem leaves aqueous extracts was subsequently tested by contact application. Treated adult *C. maculatus* of the mung bean line were found to be significantly (P<0.05) more susceptible to the botanical extract than those of the cowpea line. It was thus concluded that cowpea and mung bean seeds are more suitable for the emergence of adult *C. maculatus* than soybean seeds and that its management in storage with neem leaves aqueous extract should be done bearing in mind that the type of legume being treated might influence the effectiveness of the botanical insecticide.

Keywords: ***Callosobruchus maculatus,*** **neem aqueous extracts, cowpea, mung bean, soybean.**

INTRODUCTION

Grains of legume like cowpea (*Vigna unguiculata* (L.) Walp), chickpea (*Cicer arietinum* L.), mung bean (*Vigna radiata* (L.) Wilczek), pigeon pea (*Cajanus cajan* (L.) Huth), soybean (*Glycine max* (L.) Merr.) and bambara groundnut (*Vigna subterranean* (L.) Verdc.) are important sources of plant protein, minerals and vitamins which are essential to humans and animals (Osekre and Ayertey, 2002; Mian, 2006; Lewis et al, 2005; Banaszkiewicz, 2011). Grain legumes are cultivated in large quantities and consumed as an alternative or supplement to animal proteins especially in places where the latter is expensive or inadequate (Ofuya, 2001). Most of the world's grain legumes are produced in Africa and Asia (FAO, 1994) by subsistence farmers who store their grains in traditional structures (Nukenine, 2010). These are then bagged for onward transport to urban areas where they are again stored in ware houses before being sold in the open market.

In the course of storage, most of the grains become decimated by storage bruchids mainly *Callosobruchus maculatus* (F.) (Kashiwaba et al., 2003). This cosmopolitan field to store insect pest prefers to attack and parasitize stored cowpea as its main host (Atwal and Dhaliwal, 2005). It can however also infest several other types of stored legume grains such as chickpea, bambara nut, mung bean, groundnut and soybean (Ekeh et al., 2013) to varying degrees. When *C. maculatus* attacks stored legume seeds, they deface them with eggs and holes and invariably cause a reduction in their market and nutritional value (Musa, 2012).

Conventional insecticides like pirimiphos-methyl, chlorpyriphos-methyl, deltamethrin, fenitrothion and malathion as well as fumigants like aluminium or magnesium phosphide, methyl bromide and carbondisulfide are common protectants for grains in storage (Flanders, 2016). Their use has over the years been accompanied by several problems that include environmental pollution (Assad *et al*., 2006), toxicity to non-target organisms (Dennis, 1981) and insecticide residues in food (Shazali *et al.,* 2003). Consequently, botanical based insecticides have been recommended as alternatives to conventional insecticides for grain protection because they are more innocuous to the environment (Uddin II and Adesiyun, 2012), less toxic to non-target organisms (Ojumoola et al., 2017) and safer for grain treatment (Adedire, 2003). Neem (*Azadiractha indica* A. Juss) has been widely reported to possess a number of biological, medicinal and pesticidal (Biswas et al., 2002; Uddin II and Abdulazeez, 2013; Chudasama et al., 2015).

Insect development is influenced by diet type amongst other factors (Tefera et al., 2010). Diet is also capable of increasing or decreasing insect's susceptibility to insecticides (Liang et al., 2007; Gbaye et al., 2011). Most works on how insect pests' susceptibility to insecticides is influenced by diet type (Arthur, 2000a; Athanassiou et al., 2009; Gbaye et al., 2011, 2012) have focused more on synthetic insecticides than on botanical insecticides. This study therefore seeks to investigate the influence of three different legume seeds (cowpea, mung bean and soybean) on the emergence and susceptibility of the cowpea bruchid, *Callosobruhus maculatus* to aqueous extracts of neem (*A. indica* A. Juss) leaves.

MATERIALS AND METHODS

Experimental location

The study was conducted under ambient laboratory conditions (25-30^{0}C, 70-75% relative humidity) in the Department of Crop Protection, University of Ilorin, Ilorin, Nigeria (8.50^{0}N, 4.68^{0}E).

Legume seeds

Three legume seed types namely: *Vigna unguiculata* (IT89KD391 variety), *Vigna radiata* (NM 92 variety) and *Glycine max* (TGX 1448 variety) were used in this study. IT89KD391 is a medium sized brown and rough coated seed while TGX 1448 is medium sized with a yellow and smooth seed coat. Both were sourced from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. On the other hand, NM 92 is small sized with a green and smooth seed coat and was obtained from the Department of Agronomy, College of Crop and Soil Science, Micheal Okpara University of Agriculture, Umudike, Abia State, Nigeria. Cope and Fox (2003) have reported that in a no choice situation, female *Callosobruchus maculatus* will deposit eggs on any (seed) surface. Edde and Amatobi (2003) also reported that seed size and seed coat color have no significant effects on oviposition by the female *C. maculatus.* On receipt, the seeds were sterilized by deep freezing (< 0^{O}C) for 3 days. This was done to terminate the development of any stage of *C. maculatus* that might be in the seeds.

Insect culture

The cowpea bruchid, *Callosobruchus maculatus* was used in this study. Cultures of the insect were obtained from the insectary of the Entomology Unit of the Nigerian Stored Products Research Institute (NSPRI), Ilorin, Kwara State, Nigeria. From this initial culture, new cultures of the insect were reared in the laboratory (25-30^{0}C, 70-75% relative humidity) on a local susceptible variety of cowpea in 1L plastic jars covered with muslin cloth. First generation of *C. maculatus* adults that emerged from these were used in the experiment.

Botanical insecticide

Fresh neem leaves were harvested from neem trees in the study area. These were air dried for 24 hours after which 4kg of the leaves were soaked in 10L of water for another 24 hours. The leaves were thereafter removed and the resulting extract passed through a colander to sieve any plant debris. A total volume of 600ml of the extract was made by diluting 450ml of the stock extract solution (0.4kg/L concentration) with 150 ml of distilled water. The diluted extract solution was determined by calculation to be 30% of the initial stock solution.

Experimental procedure

Bioassay on Emergence of *C. maculatus*

The experiment was set up in a Completely Randomized Design (CRD) with three legume seed treatments namely cowpea, soybean and mung bean. Two hundred grams (200 g) of each legume type was weighed into separate 1L transparent plastic containers (11.2cmx7.5cmx11cm). This was replicated three (3) times for each type of legume seed. The difference in seed size and weight precluded the use of equal number of seeds. Thirty (30) beetles (less than 3 days old) were then introduced into each of the experimental unit in the ratio 1:2 (10 males and 20 females). The number of beetles introduced ensured that sufficient numbers of eggs were laid per seed. The sex ratio was adopted to simulate natural *C. maculatus* populations and to reduce the probability of low oviposition rate due to the introduction of unmated or infertile females. Mating and egg-laying was allowed to go on for 13 days after infestation (DAI) after which all introduced beetles (dead or alive) were removed. Emergence of adult *C. maculatus* from the egg bearing seeds in each treatment was observed daily. Emerged adults were counted per replicate and removed each day until the fourth filial (F_4) generation (a total period of 86 days).

Bioassay on Susceptibility of *C. maculatus* to Neem Leaves Aqueous Extracts

After the emergence of the F_4 *C. maculatus* beetles, 2ml of the 30% neem leaf aqueous extract concentration was introduced into petri dishes laid on the inside with filter paper (9.0mm Whatman's No 1) using a 5 ml hypodermic syringe without needle. Ten randomly selected F_4 teneral adults from each legume seed type were then introduced separately into the petri dishes. Each treatment was replicated 3 times. Fourth filial generation adults were used in the susceptibility test because they were more acclimatized to the rearing diet than adults in previous generations. Susceptibility (expressed as percentage mortality) of *C. maculatus* to neem leaves aqueous extracts was observed at 1 hour, 3 hours, 6 hours, 12 hours, 24 hours and 48 hours after setup (HAS). Beetles were considered dead when they failed to respond to repeated prodding with a laboratory forceps.

Data analysis

Data collected on adult daily emergence and percentage mortality were subjected to a one-way analysis of variance. Mean separation was done using the Tukey's Honestly Significant Difference at 5% level of significance. All statistical analyses were carried out using the IBM SPSS Version 21.

RESULTS

Table 1 shows the overall mean numbers of emerged *C. maculatus* adults from each of cowpea, mung bean and soybean over 4 filial generations. Daily emergence of 1st filial (F_1) generation adult *C. maculatus* occurred over a period of 24 days (Fig 1) with the highest (75.80) and lowest

(0.33) mean numbers recorded in cowpea and soybean respectively. The 2nd filial generation spanned 21 days (Fig 2) with significantly more adults (125.57) emerging from mung beans than from either cowpea or soybean. There was however no significant difference (P>0.05) in the overall mean number of emerged adults from cowpea and soybean in the F_2 generation. In the 3rd filial generation, a similar trend was observed with a significantly higher (P<0.05) overall mean number of adults (192.93) emerging from mung beans within 18 days (Fig 3) compared to cowpea or soybean. There was however no significant difference (P>0.05) in the overall mean number of adults that emerged from cowpea and mung bean in the 4th filial generation which spanned 23 days (Fig 4).

In all filial generations, soybean produced the least mean number of adults and that was only in the F_1 generation. There was a significant difference (P<0.05) in the mean number of emerged adults from soybean and the other legumes in all filial generations.

Table 1: Overall mean emergence of adult *C. maculatus* from three legumes in 4 filial generations

Legume Type	F_1 (24 days)	F_2 (21 days)	F_3 (18 days)	F_4 (23 days)
Cowpea	$75.80^{a} \pm 23.3$	$18.49^{b} \pm 15.1$	$59.37^{b} \pm 9.0$	$180.60^{a} \pm 21.02$
Mung bean	$41.35^{ab} \pm 11.1$	$125.57^{a} \pm 22.6$	$192.93^{a} \pm 24.8$	$157.17^{a} \pm 13.8$
Soybean	$0.33^{c} \pm 1.2$	$0.00^{b} \pm 0.0$	$0.00^{c} \pm 0.0$	$0.00^{b} \pm 0.0$

Values are means ± standard error of mean

Mean values in a column followed by the same letter (s) are not significantly different at P=0.05 according to Tukey's Honestly Significant Difference.

F_1 – 1st filial generation; F_2 – 2nd filial generation; F_3 – 3rd filial generation; F_4 – 4th filial generation

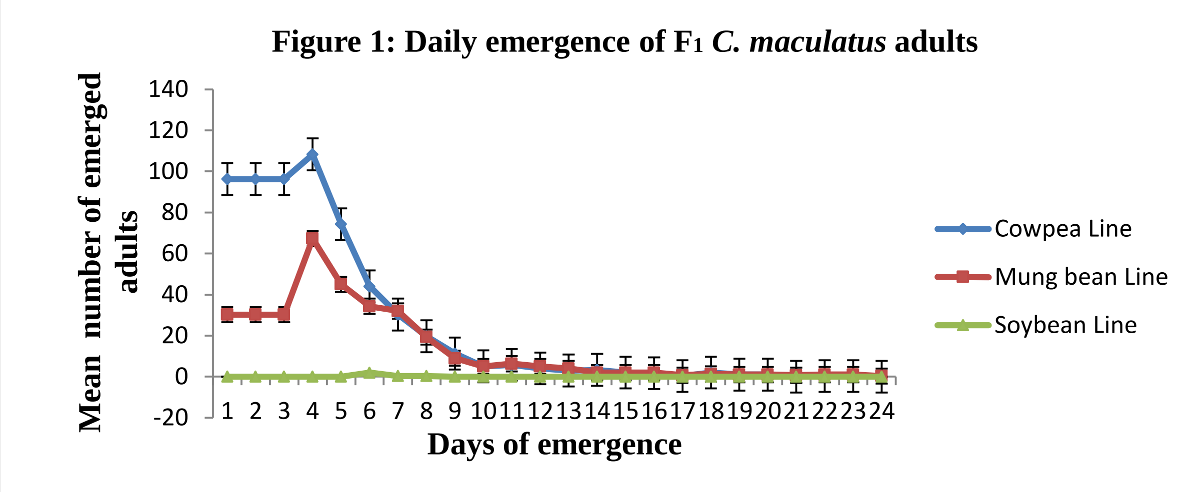


Figure 1: Daily emergence of F1 C. maculatus adults
Mean number of emerged adults
140
120
100
80
60
40
20
0
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Days of emergence
Cowpea Line
Mung bean Line
Soybean Line

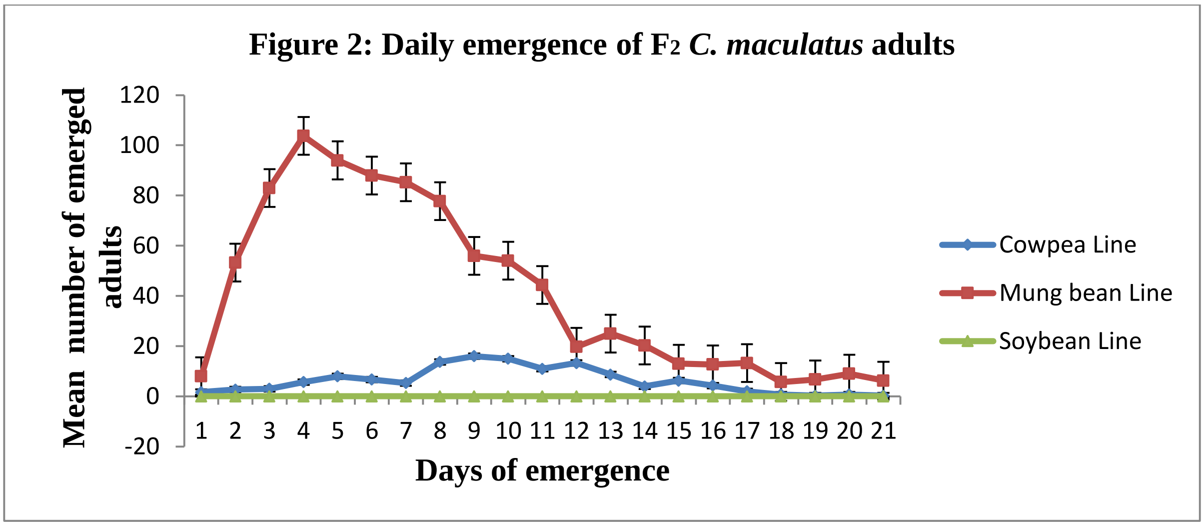
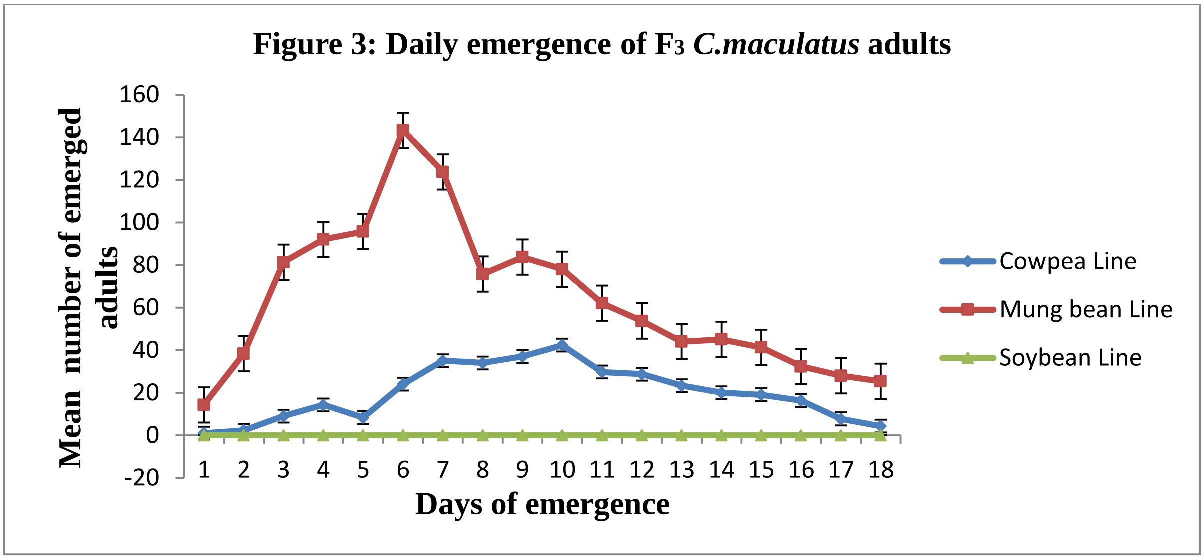
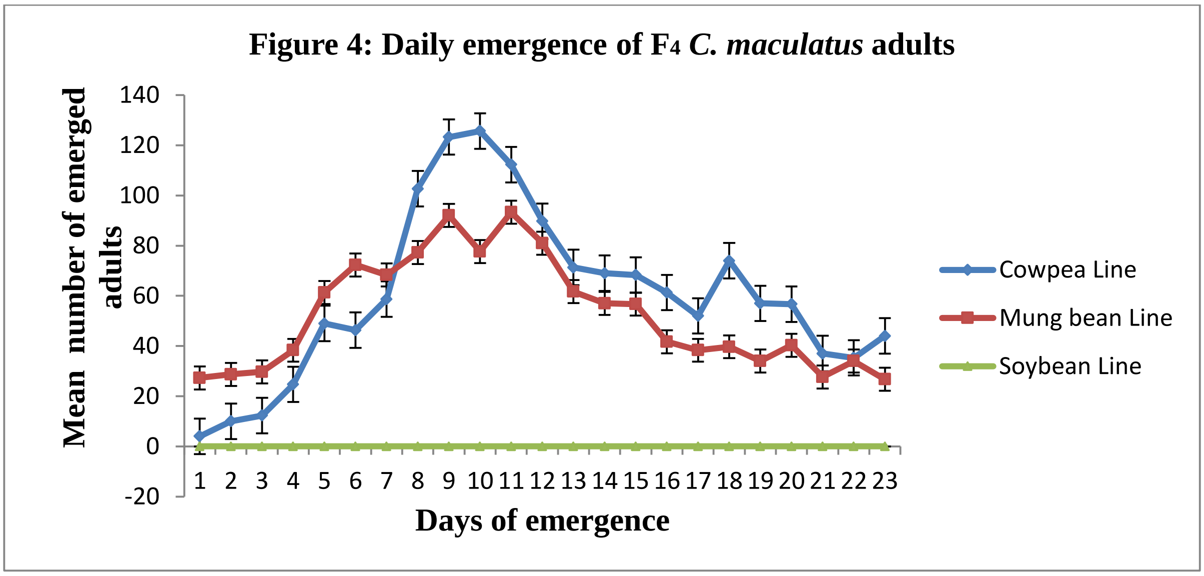


Figure 2: Daily emergence of F2 C. maculatus adults
Mean number of emerged adults
120
100
80
60
40
20
0
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
Days of emergence
Cowpea Line
Mung bean Line
Soybean Line





The effect of legume seed type on the susceptibility of adult *C. maculatus* exposed to neem leaves aqueous extract is shown in Table 2. The percentage mortality of beetles reared on cowpea was significantly ($P<0.05$) lower than that of beetles reared on mung bean after 3, 6, 12, 24 and 48 hours of exposure to the botanical insecticide.

Table 2: Effect of legume seed on percentage mortality o adult *C. maculatus* exposed to neem leaves aqueous extract

	Hours of Exposure (n=10)					
Seed type	1	3	6	12	24	48
Cowpea	$0.00^{a}\underline{+}0.0$	$0.00^{a}\underline{+}0.0$	$0.00^{a}\underline{+}0.0$	$0.00^{a}\underline{+}0.0$	$0.00^{a}\underline{+}0.0$	$6.67^{a}\underline{+}6.7$
Mung bean	$6.67^{a}\underline{+}3.3$	$26.67^{b}\underline{+}8.8$	$43.33^{b}\underline{+}12.0$	$60.00^{b}\underline{+}20.8$	$83.33^{b}\underline{+}8.8$	$100.00^{b}\underline{+}0.0$

Values are means ± standard error of mean

Mean values in a column followed by the same letter(s) are not significantly different at P=0.05 according to Tukey's Honestly Significant Difference.

*No F_4 adult *C. maculatus* from soybean was included because none emerged

DISCUSSION

According to Sharma and Thakur (2014), the nutritional value of the seed host determines to a large extent the development of *C. maculatus*. Legumes like cowpea, mung bean and soybean have been reported to contain high quality protein (Khalid and Elharadallou, 2014; Mubarak, 2005; Messina, 1999) needed by organisms for proper growth and development. However, in this study, only cowpea and mung bean supported the development and emergence of *C. maculatus* for four (4) consecutive generations. A few adults emerged from infested soybean seeds in the first generation only but not in subsequent generations. Soybean is known to have a protein content that is twice that of cowpea or mung bean (Gopalan et al, 2002; Azlan *et al*., 2011; Wilson, 2004). Yet it least supported the emergence of *C. maculatus* showing that other factors that are not nutrient related may be involved.

The susceptibility of *C. maculatus* adults reared separately on cowpea and mung bean for four (4) generations to neem aqueous extracts in this study confirmed the statement of Liang et al., (2007) that the ability of an insect to withstand an insecticide can be increased or decreased by the physicochemical properties of the food on which it develops. Despite the comparable nutritional composition of cowpea and mung bean (Golpan et al, 2002; Agugo and Onimawo, 2009), a significantly higher mortality response of 4th generation *C. maculatus* adults from the mung bean line were observed compared to adults from the cowpea line indicating the operation of other factors outside of nutritional composition.

Aside from their nutritive contents, legumes are also known to contain anti-nutritional compounds (ANCs) that may be proteinous or non-proteinous in nature. Proteinous ANCs include protease inhibitors like trypsin and chymotrypsin, lectins and antifungal peptides while non-proteinous ANCs include alkaloids, tannins, phytic acid, saponins and phenols (Duranti and Gius, 1997; Ileke, 2014). Trypsins in seeds decrease their nutritional qualities by forming indigestible complexes with the dietary proteins and these limit the absorption of important amino acids (Krupa, 2008; Gemede and Ratta, 2014). Tannins are polyphenolic compounds known to also form complexes with seed proteins and thus decrease protein digestibility. They can also interfere with dietary iron absorption (Redden et al., 2005; Aletor, 2005). Phytates are the salt form of phytic acids and are ubiquitous in seeds. They occur as mono- and divalent cations and negatively impact the bioavailablity of divalent and trivalent mineral ions (Mueller, 2001).

Compared to cowpea or mung bean, soybean has higher amounts of tannins, phenols and trypsin inhibitor contents (Mubarak, 2005; Agugo et al., 2013). On the other hand, the quantity of tannins and trypsin inhibitors in mung bean is comparable to what obtains in cowpea (Sharma and Thakur 2014; Dahiya et al., 2015). The presence of these ANCs and the variation in their amounts within the seeds of the three legumes is probably responsible for the low emergence of *C. maculatus* from soybean seeds and higher susceptibility of mung bean reared *C. maculatus* to neem leaves aqueous extracts.

CONCLUSION

This study shows that stored cowpea and mung bean seeds are more suitable for the development and emergence of adult *C. maculatus* than soybean seeds and will require more attention with respect to protection in storage. The study also showed that management of *C. maculatus* in storage with neem leaves aqueous extract should be done bearing in mind that the type of legume being treated might influence the effectiveness of the botanical insecticide.

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