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INSECTICIDAL EFFECT OF FRUIT EXTRACTS FROM *Xylopia aethiopica* AND *Dennettia tripetala* (ANNONACEAE) AGAINST *Sitophilus oryzae* (COLEOPTERA: CURCULIONIDAE)

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The insecticidal and repellent activities of fruit extracts of *Xylopia aethiopica* (Dunal) A. Rich. and *Dennettia tripetala* (Baker f.) G.E. Schatz belonging to the family Annonaceae was studied against *Sitophilus oryzae* (L.), an economic, primary post-harvest pest of rice, and other cereal products. Infested rice grains (100 g) treated with 1, 2, 3, 4, and 5% (w/w) powders of both plants were evaluated for toxicity against *S. oryzae* every 24 h for 3 d, and during F_1 progeny emergence. The essential oils of both plants were also applied at 0.5, 1, 1.5 and 2 mg cm^{-2} filter paper in Petri dishes for toxicity bioassays at 24 h exposure. Repellence bioassay with 10 µL solution of essential oils on filter paper was performed in a Y-Tube airflow olfactometer. Results indicate that powders of both plants significantly ($P < 0.001$) caused adult weevil mortality and a reduction in F_1 progeny emergence than the control. Essential oils were also significantly (P < 0.001) adulticidal to *S. oryzae* after 24 h with the highest dose (2 mg cm^{-2}) producing 100% mortality respectively. Similarly, both male and female weevils significantly avoided the test arm compared to the control arm in the Y-Tube olfactometer repellence tests. These results suggest that *X. aethiopica* and *D. tripetala* natural extracts have potential for use as part of integrated pest management of stored product protection against *S. oryzae*.

Key words: Essential oils, powders, repellence, toxicity, Y-Tube olfactometer.

Among the cereals, rice, *Oryza sativa* L. (Poaceae), is the most important staple food supplying energy and protein requirements for nearly half of the world's population. The harvested crop is often stored to provide future food, income, feed, raw material for industries and planting. However, during storage, losses of cereals such as rice grains can range from 10 to 20% of overall production, and a primary factor in these losses is the depredations of stored-product insect pests (Rajendran and Muralidharan, 2001; Phillips and Throne, 2010). The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), is an important pest of stored cereals and processed cereal products in tropical and warm temperate regions of the world (Dal Bello *et al*., 2001; Rees, 2004).

But concerns regarding the continuous use of conventional insecticides, including difficulties in registration of those insecticides in some countries, development of resistance by pest organisms, persistence in the environment, elimination of beneficial organisms, mammalian toxicity, residues on food (Daglish, 2004; Sousa *et al*., 2009), higher cost of crop production and technical difficulties at times of application, have led researchers to the evaluation of new reduced-risk insecticides to control stored-product pests. Low human and eco-toxicity have been the subject of much attention for alternative control measures of stored-product pests protection.

Different spice and herbal plant products in the form essential oils (EO), powders, pellets, extracts or distillates could be harnessed as potential toxicants, deterrents, antifeedants, repellents, and fumigants for exclusion of stored-product pests from grain, and have been used, but low toxicity has obtained much attention for alternative control measures of stored-product pests. Diverse essential oils (EOs) and other plant products have been used.

The African pepper or spice tree, *Xylopia aethiopica* (Dunal) A. Rich. (Annonaceae), is an important, evergreen, medicinal plant widely distributed in West Africa, and concoctions prepared from its morphological parts are used in traditional medicine for the treatment of skin infections, candidiasis, cough, fever, dysentery and stomach ache (Okigbo *et al*., 2005). Extracts from *X. aethiopica* have been reported to exhibit anti-bacterial and antifungal (Okigbo *et al*., 2005), mosquito repellent (Adewoyin *et al*., 2006) and termite antifeedant (Lajide *et al*., 1995) activities. Pepper fruit, *Dennettia tripetala* (G.) (Baker f.) G.E. Schatz (Annonaceae), is a woody forest and spicy plant, cultivated in Southern states

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of Nigeria, where the leaves and fruits are used in combination with other herbs for the treatment of cough, infantile convulsion, and worm infestation (Ejechi and Akpomedaye, 2005). *Dennettia tripetala* extracts have also been reported to exhibit insecticidal (Egwunyenga *et al*., 1998), and antifungal (Nwachukwu and Osuji, 2008), properties.

The objectives of this study were to investigate the insecticidal and repellent properties of *X. aethiopica* and *D. tripetala* fruit powders and essential oils against *S. oryzae* of stored rice.

MATERIALS AND METHODS

Insects and plant materials

The study was conducted between June and December 2010 in the laboratory of the Department of Crop Science, University of Calabar, Nigeria. *Sitophilus oryzae* used in this study was obtained from laboratory cultures maintained on untreated rice at 25 ± 1 ºC, 65-70% relative humidity. Three day old male and female adult weevils were used for the experiments.

Untreated rice was purchased from Obudu Local Government Foodstuff market, and matured ripe fruits of *X. aethiopica* and *D. tripetala* were bought from Marian Foodstuff Market Calabar. The identity of plant materials was confirmed in the Department of Crop Science, University of Calabar. The fruits were rinsed with tap water and oven dried at 30 ºC for 3 d. Dried fruits were milled into powder with a mechanical blender, weighed into several doses and sealed in transparent plastic containers until needed.

Extraction of essential oils

Powders of *X. aethiopica* and *D. tripetala* (150 g) were separately extracted with 250 mL distilled hexane for 24 h at room temperature. The extract was filtered through filter paper and the residue re-extracted for another 24 h before filtration. Hexane was evaporated under vacuum using a rotary evaporator at room temperature to obtain the essential oils (EO), which were sealed in glass vial and stored in the refrigerator until needed.

Insecticide effect of plant powders

Rice seeds (100 g) were treated with 1, 2, 3, 4, and 5% (w/w) *X. aethiopica* or *D. tripetala* powders in separate transparent plastic containers, with screwed lids to avoid escapes with a drilled hole covered with nylon mesh for ventilation, while the controls received no plant powders. Twenty pairs of 3-d old *S. oryzae* adults were set into each container-treatment for mating and oviposition for 6 d. The plastic containers had their covers drilled with holes to facilitate air circulation. They were then covered with nylon mesh and their perforated lids screwed in place to facilitate confinement of the weevils. Each treatment was replicated six times and laid out in a completely randomized design on the laboratory bench. A mortality count was carried daily for 3 d after treatment (DAT) by sieving out the contents into a clean white tray and counting the number of dead insects. Each time a count was done; dead individuals were discarded while live ones were returned to their respective treatments. Weevils were considered dead if they did not react or move when lightly probed with a dissecting needle. After 6 d all live and dead weevils were removed and discarded, and the seeds kept aside to wait for F_1 progeny emergence. The emerged F_1 progeny was removed and counted to give a measure of daily productivity. The experiment was ended after 3-mo when no more adult weevils emerged from any treatment.

Adulticidal activity of essential oils

Bioassay on the toxicity of *X. aethiopica* or *D. tripetala* EO against adult *S. oryzae* was similar to the method described in Bekele and Hassanali (2001) in Pyrex glass Petri dishes (10 cm diameter). Different doses of each EO were dissolved in 1 mL distilled hexane and delivered to the Petri dishes pre-lined with Whatman N° 1 filter paper. Essential oil solutions were applied at doses of 0.5, 1.0, 1.5, and 2.0 mg cm^{-2}, while the control received only hexane. Thirty seconds was allowed for the solvent to evaporate, and 10 pairs of *S. oryzae* adults were introduced into each Petri dish. The Petri dishes were sealed and maintained in the laboratory for 24 h at ambient temperature and relative humidity. All treatments were replicated six times, and a count of dead weevils was made after 24 h.

Repellence of essential oils

Solutions of the EO in distilled hexane (1 mg mL^{-1}) were prepared for the repellence bioassay. Bioassays were conducted using a glass Y-tube olfactometer (2 cm internal diameter [id], 16 cm stem length, 14 cm arms length) in a black cage set up (60 × 60 × 80 cm) consisting of a wooden frame covered with black cardboard paper to avoid external visual cues. Uniform illumination was provided by a fluorescent light tubes positioned about 30 cm above the Y-tube junction. A pump provided an air stream that was passed through an activated charcoal filter, and then split through two flow meters at 100 mL min^{-1} to each of the olfactometer arms. Each airstream then passed through a glass side arm containing 10 μL test stimuli (*X. aethiopica* or *D. tripetala* EO) or 10 μL solution of solvent (distilled hexane) or a piece of filter paper. Adult *S. oryzae* were kept singly and starved for 24 h before used for the experiments. Ten weevils were used for each bioassay, and each experiment was replicated 12 times. After each replicate, the olfactometer was rotated 180° to avoid directional effects. During the bioassay, each weevil was introduced individually into the base of the Y-tube and the numbers of weevils entering each arm were recorded. An insect was deemed to have made a final choice if it spent 15 s in motion beyond the choice line without crossing back out again or if it entered an arm but did not return back within 30 s to enter the opposite arm. An insect was tested only once, while odor sources and olfactometers changed after every replication. All bioassays were conducted between 09:00 and 12:00 h, but with treatments alternated, to avoid any effect of the time of the day on the response.

Statistical analysis

Data obtained from contact toxicity tests of plant powders and EO were subjected to ANOVA followed by comparison of means by Tukey's 95% simultaneous confidence intervals. The Y-tube olfactometer data were analyzed using a paired t-test after ensuring that data were normally distributed (GenStat Version 13 statistical software, VSN International Ltd., Hemel Hempstead, UK). Results of toxicity test were converted to percentages after ANOVA.

RESULTS AND DISCUSSION

Powders of *X. aethiopica* and *D. tripetala* fruits at various concentrations on treated rice grains showed significant ($P < 0.001$) mortality of adult *S. oryzae* than the controls. The highest mean number of weevil mortality recorded in *X. aethiopica* treated seeds occurred at 3rd day (13.17 at 5% w/w) (Table 1), and on *D. tripetala* treated seeds (11.83 at 5% w/w) (Table 2). The insecticidal activity of both plant powders increased with increased concentrations and days of exposure (Tables 1 and 2). Data from this study also showed that mean number of emerged adult weevils in control treatments were significantly ($P < 0.001$) higher than F_1 progeny in treated seeds (Table 3). The highest mean number of F_1 progeny produced in *X. aethiopica* treated seeds was observed in the control (140.50), and the least in 5% powder treated seeds (31.83). Similarly, with *D. tripetala* treatment, 5% test had the least mean number of emerged adults (41.17) and the control the highest mean number of 149.67 weevils.

Results from the toxicity of EO showed that *S. oryzae* was slightly susceptible to both plant oils at a minimum concentration of 0.5 mg cm^{-2} but highly susceptible at 1 mg cm^{-2} concentration resulting to 84.17 and 83.33% mortality for *X. aethiopica* and *D. tripetala* treatments respectively (Table 4). Complete control (100% mortality) of the weevil was achieved with 2 mg cm^{-2} dose treatment of both plants EO after just 24 h exposure. However, no mortality was obtained in the untreated control. Results of the repellence behavioral tests of EO against *S. oryzae* are shown in Figure 1. Both male (Figure 1a) and female (Figure 1b) weevils showed significant ($P < 0.001$) avoidance behavior to *X. aethiopica* and *D. tripetala* treated olfactometer arms which contained 10 µL EO solution compared to the controls with 10 µL solvent (hexane).

Plant natural products that constitute effective safer alternatives to synthetic insecticides without producing adverse effects on the ecosystem have been tested in the management of stored-product pests (Isman, 2006; Ukeh *et al*., 2009; Mao and Henderson, 2010). In this study, we evaluated the insecticidal and repellent properties of *X. aethiopica* and *D. tripetala* powders and EO against *S.*

Table 1. Dose dependent toxicity of *Xylopia aethiopica* powder on *Sitophilus oryzae* adult mortality at 24, 48, and 72 h post treatment under laboratory conditions.

	Mean (± SE) mortality of *S. oryzae*		
Treatments (%)	24 h	48 h	72 h
0	0.00 ± 0.00e	1.00 ± 0.22e	1.50 ± 0.22f
1	2.83 ± 0.31d	3.00 ± 0.37d	2.33 ± 0.21e
2	8.17 ± 0.31c	8.67 ± 0.21c	8.83 ± 0.54d
3	9.33 ± 0.21b	10.17 ± 0.31b	10.82 ± 0.31c
4	9.83 ± 0.31b	11.17 ± 0.40a	11.83 ± 0.31b
5	11.50 ± 0.43a	12.00 ± 0.52a	13.17 ± 0.48a
P	0.001	0.001	0.001
LSD (0.05)	0.74	0.85	0.88

Means in the same column followed by the same letter are not significantly different according to Tukey's test ($P > 0.05$).
SE: Standard error of the mean.

Table 2. Dose dependent toxicity of *Dennettia tripetala* powder on *Sitophilus oryzae* adult mortality at 24, 48, and 72 h post treatment under laboratory conditions.

	Mean (± SE) mortality of *S. oryzae*		
Treatments (%)	24 h	48 h	72 h
0	0.50 ± 0.22e	0.83 ± 0.31d	1.17 ± 0.31e
1	2.00 ± 0.26d	2.67 ± 0.33c	3.00 ± 0.52d
2	6.83 ± 0.48c	7.33 ± 0.56b	7.67 ± 0.49c
3	8.00 ± 0.26b	9.33 ± 0.21a	9.83 ± 0.40b
4	8.67 ± 0.21b	10.33 ± 0.67a	11.17 ± 0.48a
5	10.17 ± 0.40a	10.33 ± 0.79a	11.83 ± 0.79a
P	0.001	0.001	0.001
LSD (0.05)	0.81	1.25	1.24

Means in the same column followed by the same letter are not significantly different according to Tukey's test ($P > 0.05$).
SE: Standard error of the mean.

Table 3. Efficacy of *Xylopia aethiopica* or *Dennettia tripetala* powders on *Sitophilus oryzae* progeny emergence at 3-mo post treatment.

	F_1 progeny emergence (mean ± SE)	
Doses (% w/w)	*X. aethiopica*	*D. tripetala*
0	140.50 ± 8.31a	149.67 ± 11.40a
1	118.30 ± 3.94b	125.17 ± 3.68b
2	68.83 ± 1.64c	75.17 ± 2.98c
3	51.00 ± 1.53d	56.50 ± 2.39d
4	40.17 ± 1.05e	48.00 ± 2.09de
5	31.83 ± 1.89e	41.17 ± 2.54e
P	0.001	0.001
LSD (0.05)	9.48	12.23

Means in the same column followed by the same letter are not significantly different according to Tukey's test ($P > 0.05$).
SE: Standard error of the mean.

Table 4. Efficacy of *Xylopia aethiopica* or *Dennettia tripetala* essential oils on the mortality of *Sitophilus oryzae* adults at 24 h exposure under laboratory conditions.

	Mean mortality (%) ± SE	
Essential oils (mg cm^{-2})	*X. aethiopica*	*D. tripetala*
Control	0.0 ± 0.00d	0.0 ± 0.00d
0.5	21.67 ± 1.67c	14.17 ± 2.01c
1	84.17 ± 2.71b	83.33 ± 1.67b
1.5	98.33 ± 1.05a	97.50 ± 1.12a
2	100.00 ± 0.00a	100.00 ± 0.00a
P	0.001	0.001
LSD (0.05)	3.59	3.03

Means in the same column followed by the same letter are not significantly different according to Tukey's test ($P > 0.05$).
SE: Standard error of the mean.

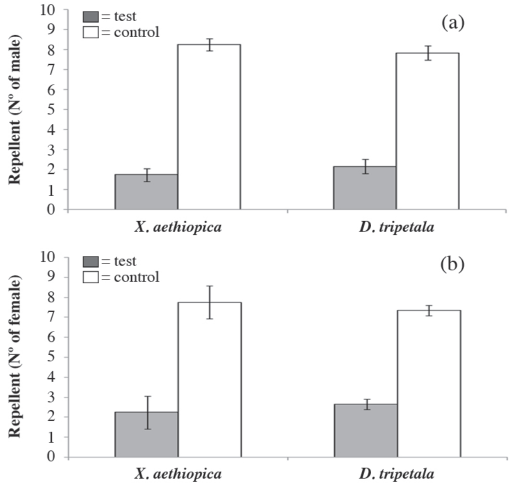

Figure 1. Mean (± SE) repellent effect of *Xylopia aethiopica* or *Dennettia tripetala* essential oils against male (a) and female (b) *Sitophilus oryzae* adults in a Y-Tube olfactometer (n = 12).

oryzae, under laboratory conditions. Results showed that powders of both herbal plants are toxic to the rice weevil by inhibiting adult survival and suppressing F_1 progeny emergence. Toxicity tests of the EO also showed that the oils were most effective with 100% of the insects killed at the highest doses of 2 mg cm^{-2} at 24 h. Y-tube repellence bioassays using male and female *S. oryzae* unequivocally produced positive results as both sexes preferred the control to the test olfactometer arm. The mode of action of both plant powders against *S. oryzae* was not studied, but it could be the pungent odors prevented the weevil from normal feeding and oviposition, or higher doses of the powders may have blocked the insect spiracles resulting to suffocation and death (Oparaeke and Kuhiep, 2006). These results are in agreement with the reports of Lajide *et al*. (1995) that 1% crude extract of *X. aethiopica* fruit oil exhibited strong antifeedant activity against workers of the subterranean termite, *Reticulitermes speratus*. The repellent activity of *X. aethiopica* fruit oil against *Aedes aegypti* mosquito, the carrier of deadly diseases such as yellow fever, dengue fever, filariasis, and encephalitis has also been demonstrated (Adewoyin *et al*., 2006). On *D. tripetala*, Egwunyenga *et al*. (1998) reported the repellent activity of the seed powder and solvent extracts against larvae and adults of the leather beetle, *Dermestes maculatus* (F.) Inyang and Emosairue (2005) also reported that aqueous extracts of *D. tripetala* seed elicited antifeedant and repellent activity against the banana weevil, *Cosmopolites sordidus* (Germar).

Studies on the insecticidal, antifeedant, and repellent effects of other plant extracts against *S. oryzae* have been documented from other published papers. For example, Park *et al*. (2003) reported that *Acorus gramineus* (Araceae) rhizome-derived extracts elicited 70 and 90% mortality against *S. oryzae* adults at 0.064 and 0.255 mg cm^{-2} at 4 d after treatment. Insecticidal and fumigant activities of EO obtained from dry ground leaves of *Artemisia scoparia* (Asteraceae) against *S. oryzae*, *C. maculatus*, and *Tribolium castaneum* (Herbst) have been demonstrated (Negahban *et al*., 2006). Similarly, the fumigant and repellent effects of *Ocimum gratissimum* (Labiatae) oil and its constituents against *S. oryzae*, *T. castaneum*, *Oryzaephilus surinamensis* (L.), and *Rhyzopertha dominica* (F.) have also been reported. The efficacy of *O. gratissimum* oil was influenced by concentration and duration of exposure after treatment (Ogendo *et al*., 2008). Omar *et al*. (2007) showed that extracts from the bark of *Lansium domesticum* (Meliaceae) had caused significant feeding inhibition of *S. oryzae* under laboratory conditions. In this study, the repellence of *X. aethiopica* or *D. tripetala* EO against *S. oryzae* is also in agreement with Yoon *et al*. (2007), who previously reported the repellent efficacy of *Carum carvii* (Umbelliferae) and *Citrus paradise* (Rutaceae) oils against *S. oryzae* in a T-tube olfactometer. Essential oil from the seed of *C. carvii* also exhibited toxic fumigant activity against *S. oryzae* at a dose of 1 μL mL^{-1} of volume on 2 cm filter paper disc (López *et al*., 2008).

Plant products including EO and their constituents are a mixture of biologically active substances or secondary metabolites of defensive nature that have been broadly studied for pest management purposes (Isman, 2006; Pavela, 2011). Essential oils are a very complex mixture of terpenes, sesquiterpenes, their oxygenated derivatives and other aromatic compounds. The chemical composition of EO may vary among plants, species, chemotype, climate, soil conditions, geographical location and the method of extraction (Langenheim, 1994; Wang and Lincoln, 2004; Nerio *et al*., 2010; Zapata and Smagghe, 2010). The mechanism of action of EO and their chemical components could affect the biochemical processes and disrupt the endocrine balance of insects (Reynolds, 1987; Rattan, 2010). The action of EO may also produce neurotoxicity in insects giving symptoms similar to those produced by organophosphates and carbamates insecticides (Isman, 2000; Ukeh and Umoetok, 2011), which are often characterized by hyperactivity, hyper-excitation leading to rapid knock down and immobilization (Enan, 2001; Rattan, 2010). Several EO and their components have been shown as reversible inhibitors of acetylcholinesterase (AChE) (Ryan and Byrne, 1988). Acetylcholinesterase hydrolyses acetylcholine (Ach), to choline and acetate thereby regulating nerve impulse transmission across cholinergic synapses (Siegfried and Scott, 1990; López and Pascual-Villalobos, 2010). Octopamine (OA), a multifunctional biogenic amine which plays key roles as neurotransmitter, neuromodulator, and neurohormone in invertebrates is another target of EO. The acute and sublethal behavioral effects of EO act by blocking octopamine receptors (Enan, 2005). The chemical constituents of *X. aethiopica* have been identified as diterpenes (Ngouela *et al*., 1998), diterpenes, phenolic amides and lignanamides (Lajide *et al*., 1995), monoterpenes, sesquiterpenes, oxygenated terpenes, and aromatic hydrocarbons (Boyom *et al*., 2003). Methanolic extract of the roots of *D. tripetala* isolated by chromatographic techniques resulted to the chromone alkaloid dennettine, three phenanthrene alkaloids identified as uvariopsine, stephenanthrine, and argentinine and a simple phenolic compound vanillin (López-Martin *et al*., 2002). Data from this study clearly indicate that *X. aethiopica* and *D. tripetala* powders and EO elicited direct adult mortality, antifeedant behavior, reduced F_1 progeny emergence, and repellence of *S. oryzae* under laboratory conditions.

CONCLUSIONS

Based on the results obtained in the current study, it may be concluded that *X. aethiopica* and *D. tripetala* plant materials have a broad spectrum of activity against *S. oryzae*, and the extracts could have potentials as bioinsecticides in stored-product protection. However, since plant products volatilize quickly in the environment and do not persist for longer duration unlike synthetic pesticides, there could be a need for re-application to obtain the desired results. The efficacy of plant-based pesticides could also be enhanced when dissolved or mixed with a slow-release fixative material or carrier such as starch or liquid paraffin, and incorporated as an integral part of integrated pest management system especially at a small-scale farmer level.

Efecto insecticida de extractos de fruta de *Xylopia aethiopica* y *Dennettia tripetala* (Annonaceae) contra *Sitophilus oryzae* (Coleoptera: Curculionidae). La actividad insecticida y repelente de los extractos frutales de *Xylopia aethiopica* (Dunal) A. Rich. y *Dennettia tripetala* (Baker f.) G.E. Schatz pertenecientes a la familia Annonaceae fueron evaluados contra *Sitophilus oryzae* (L.), plaga primaria de importancia económica en poscosecha de arroz y otros cereales. Granos de arroz (100 g) infestados tratados con polvos de ambas plantas al 1, 2, 3, 4, y 5% (p/p) fueron evaluados para la toxicidad contra *S. oryzae* cada 24 h por 3 d y durante la emergencia de la progenie F_1. Los aceites esenciales de ambas plantas también fueron aplicados en papel filtro a 0,5; 1; 1,5 y 2 mg cm^{-2} en cajas de Petri para bioensayos de toxicidad con exposición de 24 h. Bioensayos de repelencia con 10 µL de solución de los aceites esenciales impregnados en papel filtro fueron realizados en un olfatómetro de flujo de aire Y-Tube. Los resultados indican que los polvos de ambas plantas causan una mortalidad significativa de estos insectos ($P < 0,001$) y una reducción en la emergencia de la progenie F_1 con relación al control. Los aceites esenciales también mostraron un efecto adulticida significativo ($P < 0,001$) después de 24 h con la dosis más alta (2 mg cm^{-2}), produciendo un 100% de mortalidad. De igual manera, tanto hembras como machos de *S. oryzae* evitan el tratamiento en una proporción significativamente mayor al control en los ensayos de repelencia en el olfatómetro Y-Tube. Estos resultados sugieren que los extractos naturales de *X. aethiopica* and *D. tripetala* tienen un uso potencial en el manejo integrado de plagas de productos almacenados contra *S. oryzae*.

Palabras clave: aceites esenciales, polvos, repelencia, toxicidad, olfatómetro Y-Tube.

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