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# Use of Timbó (*Derris* and *Deguellia*) to Control Agriculture Pests

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## ABSTRACT

*The plant species known as timbó belongs to various genera (Deguellia, Derris, Thephrosia, Millettia, Serjania, etc.) and several families (Fabaceae, Papilionaceae, Sapindaceae, and Compostae Cariocaraceae, etc.). In South America, these plants are known by various names: timbó, tingui and cunambi (Brazilian Amazon); cube and barbasco (Peru and Colombia), haiari (Guyana), nekoe (Suriname); it is traditionally used by the indigenous people to catch fish. From 1880 to 1940, pest control using extracts and oils from the plant was at its peak, and the timbó species was one of the most important groups for this purpose. After World War II, with the creation of synthetic pesticides, the mills closed and research on the timbó crop ceased. However, due to problems caused by synthetic insecticides to the environment and humans, many scientists have now begun looking into the use of insecticide plants, including timbó, once again, in search of new alternative forms of pest control that are safe, selective, biodegradable, economically sustainable, and applicable to integrated pest control programs. The chemical composition of timbó extracts present wide qualitative and quantitative variations, with rotenone being identified as the main toxic constituent; however its effect depends on the*

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*combined action of other compounds. This chapter presents the results of research on the use of extracts and powdered roots of timbó for pest control in agriculture, emphasizing its biological effects on animals. It also contextualizes the need of more scientific efforts to determine the best combination of compounds present in the plants, in order to increase their effectiveness in pest control.*

***Keywords:*** Pest control, Deguelin, Natural insecticide, Rotenoids, Rotenone toxicity.

## Introduction

The use of synthetic insecticides is still the main tactic used for pest management (Busato *et al.,* 2006). These result in a considerable increase in production costs (Grützmacher *et al.,* 2000), and are linked to various problems, such as biological imbalance, high residual levels in foods, poisoning of applicators, and selection of resistant insect populations (Cruz, 1995; Henandez and Vendramim, 1996; Diez–Rodriguez and Omoto, 2001; Yu, 2006; Yu and MCord, 2007).

Given the problems caused by synthetic insecticides to the environment and man that have emerged in recent decades, there is a growing awareness of the need to develop technologies and products to control insect pests that are efficient, safe, selective, biodegradable, economically viable and applicable to integrated pest management programs (Viegas Júnior, 2003).

The use of alternative products presents a promising proposal for the control of insect pest species (Lima *et al.,* 2010), and involves the use of products less harmful to the environment (Vendramim, 1997), such as botanical compounds (Silva *et al.,* 2002; Bogornie and Vendramim, 2003), which theoretically have the advantage that they degrade rapidly in the environment and are more specific than the pesticides (Schmidt *et al.,* 1997; Breuer and De Loof, 1998), reducing the likelihood of generating resistant species (Valladares *et al.,* 1999; Brito *et al.,* 2004).

Plants with insecticide activity can be used in dried powder and extract forms, providing an alternative to the use of synthetic chemical insecticides, with the advantages of increased efficiency, ease of acquisition and application, rapid degradation, low toxicity to applicators, and greater safety for consumers (Faroni *et al.,* 1995; Talukder and Howse, 1995; Oliveira and Vendramim, 1999; Sousa *et al.,* 2005), adapting to the expectations of modern society in the pursuit of healthy foods, as well as programs for integrated pest management (Baldin *et al.,* 2009).

Melo *et al*. (2011) emphasize that the pest control using plants with insecticidal properties is a promising, viable and environmentally correct proposal that has gained increasing importance and attention from various scientific fields, due to their various effects on insects.

The effects of botanical insecticides on insects are variable, and include toxicity, repellant effects, sterility and deformities, modifications in behavior, the development and reduce the feeding (Arnason *et al.,* 1990, Bell *et al.,* 1990, Gusmão *et al.,* 2002).

Plants known as timbó, belonging to the genus *Derris* and *Deguelia*, are widely found in the Amazon (Costa *et al.,* 1986, Lima, 1987; Tozzi, 1998) and due to the toxins they contain (Luitgards-Moura *et al.*, 2002; Pereira and Famadas, 2004; Kotze *et al.*, 2006, Azevedo *et al.*, 2005; Alecio *et al.*, 2010, Correa, 2011; Alecio, 2012), have potential for the development of new insecticides.

There is some misinformation in the literature concerning the toxicity caused by timbó extracts. Several studies suggest rotenone to be the main toxic constituent (Crombie and Whiting, 1998; Costa *et al.*, 1986; Luitgards-Moura *et al.*, 2002; Marinos *et al.*, 2004, Azevedo *et al.*, 2007; Alecio *et al.*, 2010; Lucio *et al.*, 2011), while others point out that this substance has low toxicity when used alone, and relies on the association of other compounds to provide insecticide activity (Correa, 2011; Li *et al.*, 2011; Alecio, 2012). Meanwhile, new components have been isolated from extracts of these plants, and have shown biological effects against arthropods (Hymavathi *et al.*, 2011; Li *et al.*, 2011; Alecio, 2012; Jiang *et al.*, 2012).

Therefore, the objectives of this literature review are to gather information from research on the use of extracts and powders of timbó roots for pest control in agriculture, with emphasis on their biological effects in animals due to the chemical composition of extracts, and to contextualize the need for greater scientific efforts to determine the best combination of the compounds present in these plants, seeking to increase their effectiveness in pest control.

## History and Use of Timbó

The word timbó is of Tupi origin: *ti* meaning juice, and *mbo* meaning snake, hence, snake's juice, poisonous juice, juice that kills (Corbett, 1940). It is a plant that is known for its toxicity, especially against insects and fish, and is widely distributed in the Amazon region, both in primary forest and in cleared areas (Tozzi, 1998).

Plants that go by the name of timbó belong to several genera, including: *Deguelia*, *Derris*, *Thephrosia*, *Millettia*, *Serjania*, etc., and to different families, such as Fabaceae Papilionaceae, Sapindaceae, and Compostae Cariocaraceae (Corbett, 1940; Lima, 1987). In South America, these plants are known by various names: in the Brazilian Amazon: timbó, tingui and cunambi; in Peru and Colombia, cube and barbasco, in Guyana, haiari and in Suriname, nekoe (Pires, 1978).

According to Lima (1987) there are many species of timbó, but the most widely used in the Amazon are red timbó, *Derris urucu* (Killip et Smith) Macbride, and white timbó, *Derris nicou* (Killip et Smith) Macbride. These two species belonged to the genus *Deguelia*, and proceeded to the genus *Derris*, in a review by Francis Macbride, who placed them in the family Fabaceae.

Knowledge of the toxicity of timbó to insects is not recent. These plants have been used for pest control and fishing by the Indians and Chinese since the eighth century (Corbett, 1940). According to Lima (1987), the timbó species were already being cultivated and exploited only by the natives long before the discovery of America by Christopher Columbus. In Brazil, indigenous peoples of various regions, particularly the Amazon, use these plants to catch fish, especially on special occasions, when larger amounts of food are needed for their feasts.

In these fishing activities, the natives use the fresh roots of timbó species, which when thrown into the water and stirred, produce a milky liquid with a strong and peculiar smell. Under the action of this juice, even in very diluted form, the fish lose their balance, become stunned, and rise to the surface, where they swim uncontrollably towards the river banks and can be captured easily. In standing water, the mortality of the fish is total (Corbett, 1940; Lima, 1987).

Amaral (2004) identified the use of timbó preparations as the most common fishing method used by the indigenous Ashaninkas and Kaxinawás peoples (Wonderworker Marshall County, State of Acre, Brazil) to catch various fish species of the families Loricariidae and Curimatidae in small rivers and creeks.

Pires (1978) reports that in the Amazon, it is common to find timbó plantations in backyards or in places where there were ancient Indian dwellings, and the inhabitants of this region still use timbó roots for fishing and to kill lice in pets.

From 1880 to 1940, pest control using plant extracts and oils reached its peak. The timbó species was one of the most important for this purpose, and a large amount of research and experiments were performed with extracts of the roots of timbó, by researchers working in several countries (Caminha Filho, 1940; Corbett, 1940).

Due to the wide availability of these plants in the Amazon, and the huge market demand, the Brazilian Amazon began to export timbó roots in 1939, through the port of Belém-PA, to several countries. The United States and France were among the largest importers of this natural defense (Lima, 1947).

At that time, there were several mills operating in Brazil (Manaus-AM and Belém-PA), and the product was exported in the form of powdered timbó roots, which was used for the extraction of rotenone. Rotenone was used in the preparation of different products with widespread uses, such as pest control of crops and household infestation by insects, as well as to combat ectoparasites in domestic animals (Lima, 1987). Even today, timbó powder shipped from the port of Iquitos, in Peru, still passes through the Brazilian ports (Lima, 1947).

After World War II, with the advent of synthetic insecticides, the trade in powdered timbó roots collapsed, the mills closed, and the research activity with the crop ceased altogether (Pires, 1978). However, due to problems caused by synthetic insecticides to the environment and humans, many scientists have begun, in recent decades, to resume the research on insecticide plants seeking new alternatives forms of pest control that are safe, selective, biodegradable, economically feasible and applicable to integrated pest management programs (Viegas Júnior, 2003).

To this end, several scientists have been researching the applicability of timbó for pest control in the last few decades (Costa *et al.,* 1986; Lima, 1987; Nawnot *et al.,* 1987; Maini e Morallo,1993; Lima e Costa, 1998; Mascaro *et al.,* 1998; Tozzi, 1998; Costa *et al.,* 1999a; Costa *et al.,* 1999b; Tokarnia *et al.,* 2000; Fazolin *et al.,* 2002; Luitgards-Moura *et al.,* 2002; Pereira e Famadas, 2004; Alecio *et al.,* 2005; Azevedo *et al.,* 2005; Correa, 2006; Alecio *et al.,* 2010; Alecio, *et al.,* 2011; Correa, 2011; Alecio, 2012).

Costa *et al*. (1986) studied the effect of aqueous extract of timbó roots (*Derris urucu*) in the control of lice (*Haematopinus tuberculatus* Burmeister) in buffaloes and found that when applied by spraying, the extract was effective in combating lice, presenting the equally effective in a range of 0.25 to 2 per cent ($m.v^{-1}$), at least seven days after application. They concluded that the effectiveness of timbó is comparable to the chemicals currently used to combat this pest, with the advantage that it can be grown on the farms, its low cost, and the fact that it is readily available for use.

Maini and Morallo (1993) studied the effect of *D. elliptica* applied by spraying on snails and slugs, and found that the aqueous extract of the roots was toxic at 2,000 ppm, while the stem extract resulted in 30 per cent mortality at 10,000 ppm.

Azevedo *et al*. (2005) tested the effect of various natural products in the control of adults of *Bemisia tabaci* Gennadius biotype B (Hemiptera: Aleyrodidae) in melon (*Cucumis melo* L), under greenhouse and field conditions, and found that the timbó extract was more effective in controlling adults of these insects at the beginning of cultivation, and nymphs at the end of the cycle.

Correa (2006) determined the toxicity of the *Deguelia floribundus* Benth extract in *Toxoptera citricidus* Kirkald (Hemiptera: Sternorrhyncha: Aphidoidea) by the action of contact spray, finding $LC_{50}$ values of 1.75 per cent and 5.75 per cent ($v.v^{-1}$), respectively, for aqueous and alcoholic extracts. These concentrations were considered promising for the control of insect pests.

Alecio *et al*. (2005) evaluated the insecticidal potential of the extract of the roots of *Derris rariflora* Macbride on the maize weevil (*Sitophilus zeamais* Mots) under laboratory conditions, by means of contaminated surface intoxication on filter paper, resulting in $LC_{50}$ of 0.82 µl of extract.$cm^{-2}$.

The biocidal effect of powdered roots of *Deguelia utillis* was evaluated by Marinos *et al*. (2004) as a regulator of the *Anopheles benarroch* mosquito larvae, and it was confirmed that 3.1 g of a water-powder.$L^{-1}$ is sufficient to control 80 per cent and 90 per cent of the insect population at 24 and 48 hours respectively.

Luitgards-Moura *et al*. (2002) determined the effect on the contaminated surface (filter paper) of the alcoholic extract of *D. amazon* against larvae of *Lutzomyia longipalpis* Lutz (Diptera: Psychodidae: Phlebotominae), obtaining a mean lethal concentration ($LC_{50}$) of 21.2 per cent ($m.v^{-1}$).

Pereira and Famadas (2004) conducted laboratory tests to evaluate the efficiency of the alcoholic extract of roots of the *Dahlstedtia pentaphylla* (Taub.) Burk. against the cattle tick of *Boophilus microplus* Canestrini from Vale do Paraíba. The experiments were conducted with larvae at seven to ten days, not fed, and ticks (female engorged). The determined $LC_{50}$ were 1.65 per cent and 2.87 per cent ($m.v^{-1}$), respectively for ticks and larvae.

Costa *et al*. (1999 a) has evaluated the difference between timbó species (*D. nicou*, *D. urucu* and *D. elliptica*) collected in different regions of the Brazilian Amazon in the control of larvae of *Musca domestica* L. and observed a wide variation between different types of timbó, in terms of their effectiveness in killing insect pests, with mortalities ranging from almost zero to levels that were highly lethal to the insects. According to Costa *et al*. (1999 b), this difference in mortality in the individuals tested was due to the different amounts of rotenone contained in the root powders of each plant, as observed by means of correlation analysis, it was significant between the levels of active principle and the ability to control *M. domestica*. The different levels of rotenone found in plants of the same species of timbó originating from different regions may be due to the isolation to which people were subjected during the time of the Quaternary period, forming the so-called "forest refugia" (Costa *et al.*, 1999 a).

Gusmão *et al*. (2002) evaluated the larvicidal activity of ethanol extracts of *Derris urucu* (Leguminosae) against the mosquito *Aedes aegypti* (Diptera: Culicidae) and obtained the $LC_{50}$ of 17.6 $mg.ml^{-1}$, with 100 per cent mortality of insects at a concentration of 150 $mg.ml^{-1}$ 24 h after application of the extract. At this concentration, the larvae showed an imperfect peritrophic matrix and damage to the midgut epithelium and shed a large amount of amorphous feces, while the larvae of the control treatment produced no stools during the test period.

Ephron *et al*. (2011) evaluated the selectivity of commercial natural pesticide doses, used in organic production systems by topical application on adults of the predator *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) and found no toxicity of the commercial extract of timbó (Rotenat®), which contained at least 5 per cent of rotenone at all the tested doses (1, 3, 6 and 12 $\mu L.mL^{-1}$).

Rattanapan (2009) studied the mechanism of toxicity of the *Derris elliptica* Benth extract on *Spodoptera exigua* Hubner by the larvae immersion method, and found that the toxicity increased considerably when the substance concentration and exposure time were increased. The $LC_{50}$ were determined as: 69.15 ppm, 51.32 ppm, 46.60 ppm, at 24, 48 and 72 hours, respectively.

Dahlem *et al*. (2009) evaluated the use of alternative products to control *Diabrotica speciosa* Germar and *Sternechus subsignatus* Boheman in the culture of snap bean (*Vigna unguiculata* L. Walp) in an organic system, and found that the commercial extract of timbó (Rotenat®) containing rotenone, was not efficient for the insects.

Alecio *et al*. (2010) evaluated the insecticidal action of the extract of timbó (*D. amazonica* Killip) for adults of *C. arcuatus* Olivier, determining the $LC_{50}$ values as: 15.14 of the extract.mL-1 (by ingestion of infected leaves) and 0.45 µL of extract.cm-2 (contaminated area) and the $LD_{50}$ value as 1.44 µL of the extract per $g^{-1}$ of the insect (by topical application). The authors concluded that the extract is toxic and inhibits the supply of *C. arcuatus* at concentrations of 1 per cent ($m.v^{-1}$).

Alecio *et al*. (2011) found that *Deguelia floribundus* Benth extract caused low levels of adult mortality of *C. tingomarianus* by topical contact and by ingestion of infected leaves, but decreased insect feeding, therefore it is considered as a promising alternative for pest control.

Correa (2011) evaluated the toxicity of extracts of timbó (*Derris* spp.) and purified rotenone against *Tetranychus desertorum* (Acari: Tetranychidae) on pepper leaves, and found $LC_{50}$ values of 2 per cent and 4.4 per cent ($m.v^{-1}$) for ethanol and acetone extracts of *D. rariflora*, respectively, with 13.8 per cent ($m.v^{-1}$) for the ethanol extract of *D. floribundus* and 26.6 per cent ($m.v^{-1}$) for the acetone extract of *D. rariflora*.

## Active Compounds of Timbó

The timbó root extracts contain a wide diversity of constituents (Mendes, 1960; Mors, 1978, Costa *et al.*, 1999b; Lobo *et al.*, 2009; Jiang *et al.*, 2012). The concentration of active principles of the plants can vary according to ecological factors (nutrients, water, light and plant health), and genetic and physiological factors (Brown Júnior, 1988; Castro *et al.,* 2004; Wu *et al.,* 2008; Hymavathi *et al.,* 2011; Jiang *et al.,* 2012).

Rotenone is cited as a major constituent of timbó extract with insecticidal and acaricidal properties. However, it is commonly available in the form of powdered roots of *Deguelia* and *Derris*, containing 1-5 per cent (m.m-1) of active ingredients, or as an extract, which generally contains up to 45 per cent of rotenoids (Fang and Casida, 1999; Coll, 2005). The timbó species with the highest levels of rotenone belong to the genus *Derris* and *Deguelia*, and the highest concentration of this substance is found in the roots of these plants. Their contents can vary from 5 to 13 per cent depending on the species (Costa, 1999a; Lima and Costa, 1998).

Rotenone is a crystalline, odorless, tasteless isoflavonoid, biosynthesized by the secondary metabolite pathway, with low solubility in water and excellent solubility in chloroform, ether, acetone, carbon tetrachloride and in ethylene derivatives. In extracts of timbó, rotenone is always accompanied by other flavonoid constituents, such as deguelin, tephrosin, sumatrol, toxicarol, ellipton and malacol (Figure 14.1) (Mors, 1978). Deguelin and tephrosin also have insecticidal activities (Corbett, 1940; Cravero *et al.,* 1976, Silva *et al.,* 2002), but their toxicities for insects are respectively three and seven times lower than that of rotenone (Lima, 1987.)

Most studies with extracts of *Derris* and *Deguelia* for pest control attribute the toxicity of timbó to rotenone (Caminha Filho, 1940; Corbett, 1940; Pinto, 1953; Cravero *et al.,* 1976; Mors, 1978; Pires, 1978; Lima, 1987, Crombie and Whiting, 1998; Luitgards-Moura *et al.,* 2002; Fazolin *et al.,* 2002; Pereira and Famadas, 2004; Azevedo *et al.,* 2005, Correa, 2006; Alecio *et al.,* 2010, Li *et al.,* 2011; Lucio *et al.,* 2011).

Rotenone, present in timbó extracts, causes death of the animal through inhibition of the mitochondrial respiratory chain by blocking the phosphorylation of ADP to ATP. Fish and insects are particularly sensitive (Mascaro *et al.,* 1998). Insects poisoned with rotenone display: decrease in oxygen consumption, reduced respiration and attacks that cause seizures and ultimately lead to paralysis and death by respiratory failure (Silva *et al.,* 2002). According to Gallo *et al*. (2002), rotenone acts primarily as a potent inhibitor of the enzyme NADH-oxide reductase, the respiratory chain, and toxicity in insects is manifested by reduced heart rate, depression of respiratory movements and reduction in oxygen consumption.

Cavalheiro *et al*. (2004) found that the application of 4 mM of rotenone inhibited the respiratory chain complex I of *Candida albicans*, causing a decrease of about 30 per cent in respiratory rate (29 nmol.min$^{-1}$ $O_2$ mg of protein$^{-1}$). Colman-Saizarbitoria *et al*. (2009) observed that rotenone inhibited the respiratory chain complex I (NADH ubiquinone oxidoreductase coupling) of the crustacean *Artemia salina* Leach (Anostraca: Artemiidae) and they considered the mitochondrial inhibitor rotenone to be a classic of the respiratory chain complex I (NADH ubiquinone oxidoreductase coupling).

The cytotoxic effects of various substances on the ATP levels of insect cells (Sf9) grown "*in vitro*" were compared by Saito (2005), who found that rotenone substantially

decreased mitochondrial respiration and reduced ATP content of cells evaluated in culture medium.

Philogene *et al*. (2004) point out that rotenone is highly toxic to insects because it acts on the nervous system and mechanisms of cell respiration. Ducrot (2004) reported that rotenone can exert antifeedant, and can potentially affect biological activity or hormonal changes, causing death of the insects. According to Gosselin (1984) and Silva *et al*. (2002), rotenone is toxic to insects by ingestion and contact, thus, according to Cravero *et al*. (1976), two forms of intoxication are used to control insect pests.

Caminha Filho (1940) points out that rotenone has great value as an insecticide against crop pests, such as coccidia (Coleoptera), scale insects and aphids (Hemiptera), lice (Anoplura), wasps (Hymenoptera), butterflies and moths (Lepidoptera), etc., both as adults and in different periods of development of insects (eggs, larvae, caterpillars and pupae). It is also efficient against ectoparasites affecting domestic animals and humans, such as fleas (Siphonaptera), lice (Anoplura), ticks (Acarina) and grubs (Diptera).

Corbett (1940) found that the concentration of 1,2.10-3 g of rotenone/L in water (m.v-1) was toxic to the acarás fish (*Geophagus brasiliensis*) about half an hour after its application, and death of animals apparently occurred by respiratory paralysis, although the heartbeat continued for some time after the respiratory movements had stopped.

Pinto (1937) successfully used rotenone in powdered root form, in a soapy solution in water, against ticks, but also recommended their use in treatment of lice and fighting ticks and lice.

Saito and Luchini (1998) reported that rotenone is effective in controlling beetles and caterpillars, but its toxicity may be more or less active according to the insect species, and its action may take a little while to appear.

The antifeedant activity of several pesticides was evaluated by Nawnot *et al*. (1987) against several pests of stored products. They concluded that against *Homogyne alpina* L. rotenone obtained from *Derris elliptica* was the more effective substance.

Fazolin *et al*. (2002) obtained satisfactory results when evaluating the insecticide potential of different concentrations of rotenone extracted from *Derris urucu* against *C. tingomarianus* Bechyné in a greenhouse. The authors evaluated the effect on mortality and leaf consumption of insects, and concluded that the concentration of 0.13 per cent (m.v-1) of rotenone was effective in controlling the pest, causing significant mortality and inhibition of feeding in the insects.

Rotenone-based products were used extensively against the Colorado beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), a major potato pest in the northern hemisphere (Costa *et al.,* 1997; Cox, 2002). Rotenone has also been used to catch fish during scientific studies, which found evidence of rapid recolonization (around 3-12 days) in tide pools subjected to poisoning with this substance (Lardner *et al.,* 1993; Rosa *et al.,* 1997).

Azevedo *et al*. (2007) evaluated the effectiveness of several natural products to control *Callosobruchus maculatus* (Fab.) in cowpea (*Vigna unguiculata* (L.) Walp stored and found that commercial extract of timbó (Rotenat®), the basis of rotenone, evaluated at a concentration of 7.5 $mL.L^{-1}$, was the most effective form of control of the weevil in stored cowpea.

The oleoresin extract of yam bean (*Pachyrhizus erosus* L. Urban), at concentrations of 0.03 per cent and 0.06 per cent ($m.v^{-1}$), containing 15 $mg.L^{-1}$ of rotenone, was effective in the control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) by contact and ingestion (Lucio *et al.,* 2011).

For Tokarnia *et al*. (2000) rotenone, despite being very toxic to fish, has low toxicity to warm-blooded animals, and experimentally producing plants of this active ingredient (timbó species) has not been shown to be toxic to cattle and other livestock.

There are several factors that highlight the rotenone present in timbó extracts for pest control. It is not phytotoxic (Reynolds, 1989), it is biodegradable, and it is photosensitive when exposed to light, as it degrades within three days (Moreira *et al.,* 2005). When applied in raw form, it decomposes more quickly than nicotine and pyrethrins and is considered practically harmless to humans, due to its low concentration in the mixtures (Mariconi, 1981).

On the other hand, some studies have demonstrated the high toxicity of rotenone to arthropods (Marinos *et al.,* 2004; Azevedo *et al.,* 2007; Guirado *et al.,* 2007; Almeida, 2010; Correa, 2011; Efrom *et al.,* 2011) and other studies have pointed out that rotenone has low toxicity when used alone and that this substance depends on the association of other compounds to show toxic effects (Decker, 1942; Tyler, 1979; Costa *et al.,* 1999a; Costa *et al.,* 1999b; Correa, 2011; Li *et al.,* 2011; Alecio, 2012; Pena, 2012). Meanwhile, new constituents have been isolated and identified from extracts of timbó that have shown biological effects on arthropods (Mors, 1978; Magalhães *et al.,* 2003; Gassa *et al.,* 2005; Wu *et al.,* 2008; Lobo *et al.,* 2009; Babu *et al.,* 2010; Hymavathi *et al.,* 2011; Li *et al.,* 2011; Jiang *et al.,* 2012).

Almeida (2010) found that a defensive botanical trade, with at least 5 per cent ($m.m^{-1}$) of rotenone, showed low efficiency of insecticides on nymphs of *Euphalerus clitoriae* (Hemiptera: Psyllidae), obtaining the highest mortality rate of the insects (16.9 per cent) at the highest concentration measured (0.6 per cent $v.v^{-1}$). A similar effect was obtained by Lima *et al*. (2008) when they studied the efficiency of commercial extract of timbó (Rotenat ®) against larvae of *S. frugiperda* under natural infestation in corn grown in lowland areas. In this study, the concentration of 0.5 per cent ($v.v^{-1}$) timbó extract, with at least 5 per cent of rotenone, showed low efficiency in pest control.

The insecticidal action of several alternative products was evaluated by Guirado *et al*. (2007) for the control of *C. arcuatus* Olivier (Coleoptera: Crhysomelidae) in the sunflower crop under field conditions, confirming that rotenone, used at a concentration of 1 per cent ($m.v^{-1}$), was not effective in controlling the insects. Ephron *et al*. (2011) also evaluated doses of commercial extract of timbó (Rotenat®), containing at least 5 per cent rotenone, and observed no toxicity with topical application of the poison in adults of the predator *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae).

Correa (2011) found that the extract of the timbó *Derris rariflora* showed high toxicity against mite of the pepper *Tetranychus desertorum* (Acari: Tetranychidae), while the purified rotenone was not toxic to humans, causing only 2.5 per cent mortality in mites at the highest concentration assessed (1 per cent m.$v^{-1}$).

The biological effects of extracts of *D. urucu* and *D. utillis* were studied by Costa *et al*. (1999a) in populations of *Musca domestica* L. The authors found that the species *D. urucu* was more efficient, even with lower levels of rotenone in the roots. These authors hypothesized that other substances in addition to rotenone, could be acting to control the larvae.

Catto *et al*. (2009) evaluated rotenone in the form of *Derris nicou* root extract against *Boophilus microplus*, and found that the product was effective against larvae infestation, but produced an insufficient degree of antiparasitic activity against the tick *in vitro* and in experimentally infested animals. Neither did it show any significant decrease in the tick infestation (engorged) in the test field.

Decker (1942) reports that the crude extract of *D. elliptica* is more efficient than rotenone against insects, and that the insecticidal effect of timbó roots may be related to the joint action of rotenone with other substances present in extracts of *Derris* and *Deguelia*, including the deguelin and toxicarol. Tyler (1979) states that timbó roots may contain rotenone, deguelin, toxicarol or tephrosin, structurally similar compounds that may have insecticidal properties.

Besides rotenone, Mors (1978) has isolated other active ingredients from roots of *Derris urucu*, including a high-powered foam forming saponin, named "derrisídio". This author emphasizes that in timbó, a toxic complex of this saponin appears to have a dispersant action on the rotenone.

Mendes (1960) found significant variation in the chemical composition of 153 plant extracts of timbó of the same species (*Derris nicou* Benth), which were attributed to the different sampling sites and the age range of plants. In this study, the concentrations of the constituents of the extracts ranged from 12.5 per cent to 61.1 per cent (m.$v^{-1}$) for rotenone, from 12.7 per cent and 79 per cent (m.$v^{-1}$) for deguelin and from 2.2 per cent to 64.7 per cent (m.$v^{-1}$) to other components, enabling the author to classify the plants into two groups (A and B). Plants belonging to the group A had ¼ rotenone, $^{2}/_{4}$ deguelin and ¼ of other components, while those in group B had $^{3}/_{6}$ of rotenone, $^{2}/_{6}$ and deguelin and $^{1}/_{6}$ of other compounds in the extracts.

Alecio (2012) also observed a wide qualitative and quantitative variation of the major constituents found in extracts of roots timbó (*Deguelia* and *Lonchocarpus*), with a predominance of rotenone, deguelin, tephrosin and hidroxirotenone.

Magalhães *et al*. (2003) found three new constituents (scandenin, methyl robustato and 4',5-dihydroxy-6-3,3-dimetilalila metoxiflavanona-7) among the flavonoids isolated from Derris hatschbachii. These were identified by comparison of the spectroscopic data (NMR, NMR-2D and MS/MS).

From extracts of *Derris laxiflora*, Wu *et al*. (2008) isolated nine compounds which they characterized by spectroscopic analysis. Seven of these were considered new: O-trans-cinnamoylglutinol (1) 22β-hydroxy-12-oleanen-3-one (2), 15α, 16α-epoxyoleanen-12-3-one (3), 29-hydroxy-12-oleanene-3,22-dione (4), 22β,29-dihyroxy-12-oleanen-3-one (5), 2,3-(methylenedioxy)-4-methoxy-5-methylphenol (8) and 2,3,6-trimethoxy-5-methylphenol (9), as well as isolated from natural sources: 25-cycloartene-3,24-dione (6) and 24î-hydroxy-25-cycloarten-3-one (7).

Lobo *et al*. (2009) isolated and identified five stilbenes from the leaves of "timbó red" (*Derris rufescens* var. *urucu*): metoxilonchocarpeno 4-(1) 3,5-dimethoxy-4 '-hydroxy-3'-prenyl-trans stilbene (2), lonchocarpeno (3) 3,5-dimethoxy-4 '-O-prenyl-trans-stilbene (4) and pteroestilbeno (5). Components 2 and 4 were reported as new natural products, but compound 2 had already been mentioned as a synthetic product.

Three new dihydro-flavonoids; urucuol, denominated A, B and C and dihydroflavonol isotirumalin, were isolated and identified by Lôbo *et al*. (2009) from the ethanol extract of the leaves of *Derris urucu* (Leguminosae). Their structures were elucidated by extensive spectroscopic analysis of the 1D and 2D-NMR, UV, IR and MS data, and comparison with the literature data. The isolated compounds were evaluated for their DPPH scavenging capacity, and showed low antioxidant power when compared to the commercial antioxidant trans-resveratrol.

Babu *et al*. (2010) conducted a phytochemical study of the plant *Derris scandens* Benth (Leguminosae) using spectroscopic methods, especially 1D and 2D NMR and mass spectral analysis, which resulted in the isolation of a new derivative of isoflavones, the scandinone A, as well as 11 known compounds.

The toxicity of nine isolates compounds of *Derris scandens* was evaluated by Hymavathi Benth *et al*. (2011) for four species of insect pests in stored grains (*Callosobruchus chinensis* L., *Sitophilus oryzae* L., *Rhyzopertha dominica* L. and *Tribolium castaneum* H.), using a fumigation bioassay. The sensitivity of individuals to the compounds varied with exposure time, concentration, and insect species, reaching 100 per cent mortality in individuals after 24 h with the compounds osajin, scandinone, sphaerobioside and genistein against all insects tested, while laxifolin and lupalbigenin showed 100 per cent mortality of *T. castaneum* and *R Dominica* 72 h after the start of the bioassays.

The insecticidal action of two compounds (khayasin and 2'S-methylbutanoylproceranolide) isolates of *Xylocarpus moluccensis* Lam was compared to purified rotenone and they was assessed by ingestion of infected leaves on larvae of the coconut beetle *Brontispa longissima* Gestro (Coleoptera: Chrysomelidae), grown in the laboratory. It was observed that the two compounds were more potent than purified rotenone at a concentration of 50 mg.ml-1 (Li *et al.,* 2011).

Jiang *et al*. (2012) isolated and characterized 12 compounds (11 known and one not reported in the literature) from extracts of the aerial parts of *Derris trifoliata* Lour and evaluated their toxicity against *Artemia salina* Leach (Anostraca: Artemiidae). They identified eight constituents with significant toxicity ($LC_{50}$ between 0.06 to 9.95 mg.mL -1: rotenone, tephrosin, 12a-hidroxirotenone, deguelin, 6a, 12a-dehidrorotenone, dehidrodeguelin, 7a methyldeguelol-o-and 4'-hydroxy-7-metoxiflavanone), while the isolated novel compound showed low toxicity ($LC_{50}$ = 211.31 mg.mL -1) to the crustacean.

Alecio (2012) evaluated the biological activity of extracts from timbó (*Derris sandens* Aubl. and *Deguelia floribundus* Benth) compared to purified rotenone against adults of *Cerotoma tingomarianus* Bechyné (Coleoptera: Chrysomelidae) and *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) and verified that: the toxicity of timbó species is related to the chemical composition of the extracts, the form of exposure and the target insect species; the chemical composition of extracts timbó decisively influence the biological toxic effects on insects; rotenone, when used alone, has low toxicity but when associated with other constituents, its toxic effect is enhanced; and greater toxicity of timbó species to larvae of *S. frugiperda* is promoted when the timbó extracts are prepared with approximately $^{3}/_{10}$ of rotenone, $^{3}/_{10}$ of deguelin, $^{3}/_{10}$ of tephrosin, and $^{1}/_{10}$ hidroxirotenone and smaller amounts of other constituents. These proportions of components were considered to be most suitable for the selection of extracts from timbó, aiming at the development of biotech products to control insect pests in agriculture.

Thus, there is great interest ecological and economic in these active principles as control agents of insect pests in agriculture, or in certain plant/insect relationships (Crombie and Whiting, 1998). Further studies are needed, to define the most appropriate quantities and proportions of the active ingredients of timbó to be used for the control of each species of insect pest.

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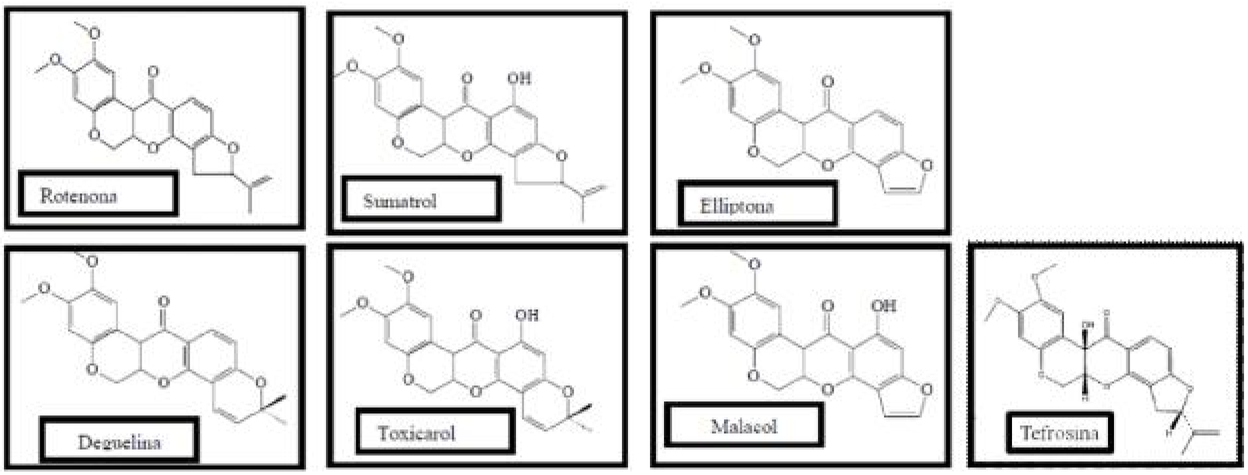


**Figure 14.1**: Rotenoids that can be found in roots of timbó (*Derris* and *Deguelia*).