

Pesticidal activity of leaves and seeds extracts of *Tephrosia vogelii* Hook. f. and *Ricinus communis* L. on insects damaging crops and stored food

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Abstract

Synthetic pesticides and biopesticides are currently used to control insect pests of crops and harvests. However, biopesticides are more highly recommended because they are environmentally friendly and safe for human health.

This study aimed to highlight the pesticidal effect of *Tephrosia vogelii* and *Ricinus communis* on four insects: *Spodoptera frugiperda*, *Aphis fabae*, *Acanthoscelides obtectus*, and *Sitophilus zeamais*. The leaves and seeds of these plants were collected from the fields and analyzed in the laboratory to perform phytochemical screening and test their toxicity to these insects.

Results showed that the phytochemistry of the two plants varied from one plant to another and from one extract to another. Aqueous extracts of fresh *T. vogelii* and *R. communis* leaves (125 g/L and 250 g/L, respectively) resulted in mortality rates exceeding 50% for *A. fabae*. Aqueous extracts of *T. vogelii* leaves were more effective than those of *R. communis* leaves against *S. frugiperda* in terms of mortality rates. LD₅₀ of aqueous extracts of *T. vogelii* leaves was 114 g/L for *A. fabae*.

Most organic extracts (4 mg/L) of both plants achieved mortality rates exceeding 60% for *A. obtectus* and *S. zeamais*. Hexane extract (4 mg/L) of *T. vogelii* leaves and seeds showed better results for treating *A. obtectus* (LD₅₀ = 1 mg/L–1.33 mg/L). Similarly, hexane extract of *T. vogelii* seeds showed better results for treating *S. zeamais* (LD₅₀=1.66 mg/ml). Therefore, it can be concluded that *R. communis* and *T. vogelii* could be alternative treatments for insect pests of crops and harvests.

Keywords: Plant extract, harvest, insecticidal effect, maceration, insect pests

Introduction

Agricultural production is seasonal, whereas consumption of agricultural products extends throughout the year. Therefore, there is a need to store harvests (Mikolo et al., 2007). Stored harvest can be used for a long period of time which may extend over one year (Rihab & Amina, 2020). But, in only few months, insect pests can wipe out stocks of food and seed intended for years. According to Ndomo et al.(2009), stocks are often attacked by several pests such as insects, fungi and rodents; but the damage caused by insects is greater. Insect pests of stocks cause significant losses by reducing stock quality and quantity (Mossa, 2016). In case of Burundi, the most harmful insects are mainly *Spodoptera frugiperda* J.E. Smith for corn crops and *Aphis fabae* Scopoli for bean crops; and for stored foods *Acanthoscelides obtectus* Say is the most harmful for stored bean seeds while *Sitophilus zeamais* Motschulsky is the most damaging stored corn kernels (INADES-Formation, 2021). Thus, to deal with this problem, farmers often resort to synthetic pesticides because of their effectiveness in controlling these insects (Redlinger et al., 1988; Rihab & Amina, 2020). However, this practice has disadvantages, both for human health and the environment (Kumar, 1991) . In fact, after the use of synthetic pesticides, there is an issue of remaining residus on crops or stored harvest which are responsible of many chronic diseases appearing each year such as cancer, accidental deaths and many nutrional poisonings (Bortoli & Coumoul, 2018). For this reason, the World Health Organisation prohibited the use of some synthetic insecticides (Rihab & Amina, 2020)

Bioinsecticides would be one of the salvific ways to solve the problems caused by both crop pests and synthetic pesticides. In the town of Kabinda, Nsomue *et al.*(2020) analysed the effectiveness of certain local plants (*Nicotiana tabacum* L., *Allium cepa* L. and L.) in controlling *S. zeamais* attack. Their study showed that maize grains treated with *Piper nigrum* had an insect attack rate of 0%. Therefore, it was suggested that *Piper nigrum* could be used as a bioinsecticide to manage *S. zeamais* attack on stored corn seeds. According to researchers (Guèye, 2012; Khani & Rahdari, 2012), essential oils of some plants should be recommended for pest control. In the highlands of West Cameroon, plants such as *Clausena anisata* and *Cupressus sempervirens*, *Capsicum frutescens*, *Chenopodium ambrosioides*, *Eucalyptus saligna*, *Lantana camara* are used as pesticides (Parh *et al.*, 1998; Tapondjou *et al.*, 2000). The pesticide's properties exhibited by some plants depend on their content in secondary metabolites. The main ones of these secondary metabolites are alkaloids, flavonoids, leucoantocyanins, quinones, saponosides, steroids, terpenes and phenolic compounds. Some of those compounds are present in the plant organs (leaf, seed, root, flour, etc) but in different concentrations. The saponosides, for instance, have already proved their defensive activity against insect pests, fungal, and bacterial pathogens and parasitic nematode infestation (Krief, 2003; Zaynab *et al.*, 2021). According to Zhang *et al.*(2020), *T. vogelii* is one of 400 species of *Tephrosia* genus that is widely studied and its toxicity of is due to the presence of rotenoids compounds mainly rotenone, deguelin, rotenolone and tephrosin which belong to isoflavonoids group. They also highlight the current use of its extracts for control of insect pests in stored grains and crop production.

In Burundi, plant species such as *Tithonia diversifolia*, *Ricinus communis*, *Vitex doniana*, *Anthocleista schweinfurtii* and *Tephrosia vogelii* are used by some farmers against crop and harvest pests. However, the efficacy and the dose required have yet to be scientifically demonstrated. The main objective of this study focusing on the biopesticidal activity of *T. vogelii* and *R. communis* on the *S. frugiperda*, the *A. fabae*, *A. obtectus* and *S. zeamais* is to contribute to the improvement and valorization of the use of pesticidal plants for a healthy population and a safe environment. Specifically, this work aims to (i) identify the presence of secondary metabolites with biopesticidal properties in aqueous and organic plant extracts, (ii) assess the efficacy of aqueous extracts of different concentrations on *A. fabae* and army worm, (iii) evaluate the efficacy of secondary metabolites of dry leaf and seed extracts as a function of organic solvent on *A. obtectus* and *S. zeamais*, (iv) determine the lethal dose (LD₅₀) of effective fresh leaf extracts and the lethal doses of effective dry leaf and seed extracts against *S. zeamais* and *A. obtectus*.

Material and Methods

Study area and sample collection

Samples of insecticidal plants were collected from Kabanga zone (Giheta commune) in middle of Burundi. The study area belongs to the central plateaus which represent 52% of the national territory. Altitude varies from 1,350 m to over 2,000 m. Average annual rainfall is around 1,200 to 1,500 mm, and from 17°C to 20°C for temperature. Soils vary in fertility, and are constantly declining due to overexploitation, erosion, poor farming practices, and high demography. Natural vegetation has completely disappeared and replaced by agroecosystems for various cultures.

The organ plants were harvested from fields where beans and banana trees were also grown. Leaves and seeds of *T. vogelii* were harvested with pruning shears at 03° 19' 15.6" S - 029° 50' 54.4" E. White leaves of *R. communis* used were collected at 03° 19' 27.6" S - 029° 51' 09.1" E, while those of red color were collected at 03° 19' 50.3" S - 029° 50' 16.3"E. The altitude varied from 1573 to 1605 m in the sampling sites. A Garmin GPS was used to record the site coordinates. Samples were collected twice for each site: in April 2022 and in January 2023. The study area, the Giheta commune, was chosen as it is one of the country communes where INADES-formation program is vulgarizing the use of biopesticides for pest control. The botanical identification was confirmed by specialists of herbarium of "Université du Burundi" where voucher specimen were deposited under the following numbers: *T. vogelii* (NGL 001) and of *R. communis* (NGL 002, white leaves and NGL 003, red leaves).

Preparation of aqueous extracts

Two hundred fifty (250) grammes of fresh *T. vogelii* and of *R. communis* (white and red leaves) were crushed. The paste obtained was poured into a bucket filled with one liter of distilled water. After 24 h, the mixtures were stirred to extract as many organic compounds as possible. The three mother extracts were filtered through a cotton cloth and some dilutions up to 62.5 g/liter were made to test the efficacy of the extract at low doses. From the 250 g/l extract, two extracts of 125 g/l and 62.5 g/l were prepared by dilution. Those two doses were chosen based on three kinds of extracts used by local farmers to control insect pests in the area under the supervision of the INADES-Formation program.

Phytochemical screening

Phytochemical screening consisted in testing the three aqueous leaf extracts of *T. vogelii* and *R. communis* for the presence of saponosides, tannins, flavonoids, glucosides and phenols.

- Saponosides: 1 ml of aqueous extract mixed with 5 ml of distilled water was rigorously shaken. A persistent, supernatant foam formation on the surface of the solutions indicates the presence of saponosides. This was the case for all aqueous extracts.
- Tannins: 1 ml of each of the extracts was mixed with 20 ml of distilled water, and heated to boiling. The solution was then allowed to cool and filtered. The resulting solution was mixed with 1 % FeCl_3 . Once the solution turns blue-black, the presence of tannins is confirmed.
- Flavonoids: 1 ml of each plant extract is mixed with 5 ml of 25 % NH_3 solution and 2 ml of 63 % H_2SO_4 solution, was boiled for 2 minutes. If the solution turns yellow, the presence of flavonoids is confirmed.
- Glucosides: a mixture of 1 ml of each plant extract, 2 ml chloroform and 2 ml of 63 % H_2SO_4 solution, was gently stirred. If the solution turns reddish-brown, a steroid nucleus is present, which is the glycone part of the glucoside.
- Phenols: 1ml of each extract was mixed with 2 drops of 2 % FeCl_3 . If the solution turns black, the presence of phenols was confirmed.

Preparation of organic extracts

Four different organic solvents in terms of polarity (n-hexane, dichloromethane, ethyl acetate and methanol) were used for extraction in open air. Based on available samples quantity, different weights of powder were used. One hundred (100) g of *T. vogelii* and *R. communis* white and red leaf powder were treated with the four organic solvents according to their polarity. A liter of each solvent was used. The same operation was carried out for the extraction of 42 g of *T. vogelii* seed powder and 150 g of *R. communis* seed powder.

A thin-layer chromatographic test of the last drop of extract was performed to ensure that the organic solvent had completely depleted from the secondary metabolites. The chromatographic plate was viewed under ultraviolet light to clearly visualize invisible spot to the naked eye. To ensure the invisibility of spots, the chromatographic plate was sprayed with sulfuric acid as a spot developer, then air-dried. The presence of black spots indicated the presence of secondary metabolites in the powder residue, in which the solvent was added to complete the extraction. In the absence of spots, the powder was air-dried to evaporate the solvent completely. The dried powder residue was then extracted with a new solvent of higher polarity than the previous one. After maceration, the solvent-secondary metabolite mixture was concentrated with a rotary evaporator under reduced pressure. A total of 20 organic extracts were obtained and stored for later use in a refrigerator set

at 4°C. For phytochemical screening of organic extracts and for each of *T. vogelii* and *R. communis*, the same protocol as for phytochemical screening of aqueous extracts, except for alkaloids and steroids, was followed.

- Alkaloids: 0.1 g of extracts was mixed with 1% hydrochloric acid. The solutions were boiled for approximately one minute. After cooling at room temperature, the solutions were filtered. Three drops of Dragendorff's solution were then added to each filtrate. The appearance of a persistent yellow color indicated the presence of alkaloids in the extract.
- Steroids: 2 ml of each extracts were mixed with 2 ml CHCl₃ and 2 ml concentrated H₂SO₄. After the solutions were stirred, the formation of a red chloroform phase and an acidic layer showing greenish-yellow fluorescence indicated the presence of steroids in the extract analyzed.

Insect pests and treatment

Spodoptera frugiperda, *A. fabae*, *A. obtectus* and *S. zeamais* are the main insect pests that destroy maize and bean crops and harvests that this work is focusing on.

Breeding and treatment of A. obtectus and S. zeamais

Mass rearing of the two insects for biological tests was carried out in the laboratory, under ambient conditions following the method of Ndomo *et al.*(2009). In 5 kg of maize and of beans kept in the laboratory for 3 months, many *A. obtectus* and *S. zeamais* appeared. For toxicity tests, 10 g of uninfected maize and 10 g of uninfected bean seeds were placed in a series of Petri dishes and coated with 1 ml of each extract at different doses (4; 2 and 1 mg/ml). Six (6) individuals randomly selected of *S. zeamais* and of *A. obtectus* were respectively placed in each Petri dish containing maize kernels and bean seeds. Beside that, for the test on *A. obtectus*, two controls have been made in Petri dishes containing bean seeds impregnated with water and 5% DMSO solution respectively, and six individuals of *A. obtectus* were inserted in each Petri dish. The same protocol was observed for the test control on *S. zeamais*.

Breeding and treatment of A. fabae

A bean plot of 70 m² area was prepared at the University of Burundi. The field was subdivided into 9 sub-plots to facilitate the control of the toxic efficiency of the extracts and 3 sub-plots were dedicated to negative control. Each sub-plot area was 4 m². One month after sowing, the *A. fabae* was observed on the underside of the bean leaves. After counting them with a magnifying glass on a bean leaf, aqueous extracts of *T. vogelli* and *R.*

communis at three different doses (250, 125 and 62.5 g/l) were sprayed into three sub-plots. Underneath the leaf, aluminum foil was laid out to collect the dead *A. fabae*. Dead aphids were counted 24 h after spraying. The insect individuals were considered dead if they remained motionless when touched with forceps.

Breeding and treatment of S. frugiperda

An attempt to rear *S. frugiperda* was unsuccessful. After a month of sowing corn, *S. frugiperda* larvae emerged and were harvested using forceps. They were placed in transparent boxes with perforated lids and sealed with sterilized cotton. Harvesting continued for two weeks. The larvae were fed fresh, cut corn leaves until they reached the chrysalis stage. To prevent cannibalism, a single larval individual was raised in each well-ventilated plastic box. When the larva, during its development, became a chrysalis, it was transferred to a test tube, sealed with a cotton ball to continue metamorphosis. After hatching, the moth pairs were placed in large plastic boxes and fed honey. Unfortunately, the pairs did not reproduce and were found dead. This may be due to the conditions of their growth that were not met. Following this breeding failure, we adopted the treatment of *S. frugiperda* directly in the field.

A maize plot, of 135 m² area, was then prepared and subdivided into 12 sub-plots, of 9 m² each, to facilitate the control of the toxic efficiency of the extracts. Three of these sub-plots were dedicated to negative control. After one month's sowing of a maize field, *S. frugiperda* larvae appeared. The maize seedlings in each plot were checked for the presence or absence of *S. frugiperda*. Once their presence had been detected, aqueous extracts of *T. vogelli* and *R. communis* at different doses (250, 125 and 62.5 g/l) were prepared and each concentration was sprayed into three sub-plots. For the toxicity tests, dead *S. frugiperda* were counted once after 24 h of spraying the field.

Data processing

Microsoft Excel 2013 was used for data capture.

Extraction yield is calculated using the following formula:

$$\text{Yield (\%)} = \frac{m_0}{m_1} \times 100$$
 where: m_0 : mass of raw extract; m_1 : mass of plant material initially weighed.

The mortality rate was calculated as follows:

$$\text{Mortality rate} = \frac{n}{N} \times 100$$
, where : n: number of dead individuals; N: total number of individuals tested in each Petri dish.

The SPSS software was used to perform statistics: Analysis of variance (ANOVA) and Student t-test to compare mortality rate of insects studied.

The LD₅₀ was calculated using the arithmetic method of Behrens and Kärber (1934).

LD₅₀ = LD₁₀₀ - $\Sigma (a \times b) / N$, where: a: difference between two successive doses; b: half-sum of dead individuals between two successive doses; N: total number of individuals in a Petri dish.

Results

*Phytochemical screening of *T. vogelii* and *R. communis* aqueous extracts*

The presence or absence of glycosides, flavonoids, tannins and phenols in the aqueous extracts of the leaves of *T. vogelii* (TVF) and those of *R. communis* with white (RCFb) and red (RCFr) leaves is shown in Table 1.

Table 1: Results of phytochemical screening of aqueous extracts

Aqueous extracts	Secondary metabolites				
	Glycosides	Saponosids	Flavonoids	Tannins	Phenols
TVF	+	+	+	+	+
RCFb	+	-	+	+	+
RCFr	+	-	+	+	+

Legend: (+): Presence of secondary metabolite; (-) : Absence of secondary metabolite

*Phytochemical screening of organic extracts from leaves and seeds of *T. vogelii* and *R. communis**

The methanol leaf extracts of *T. vogelii* and *R. communis* red leaves and the hexane extract of *R. communis* white leaves contained alkaloids, saponosides, flavonoids, steroids, tannins, phenols and glycosides (Table 2). Glycosides were present in almost all *T. vogelii* organic extracts except in its hexanic seed extract, while there are the only secondary metabolites present in the hexanic extract of *T. vogelii* leaves (Table 2). The two secondary metabolites, flavonoids and steroids, are found in all organic extracts from *R. communis* red leaf, regardless the extraction solvent used and in the hexanic and ethyl acetate extracts of *R. communis* white leaf seeds. (Table 2).

Finally, for *R. communis* white leaf seed extracts, regardless the type of organic extraction solvent used, the screening showed the presence of alkaloids.

Table 2: Results of phytochemical screening of organic extracts from leaves and seeds of both plants

Extract types	Secondary metabolites						
	Alkaloids	Saponosides	Flavonoids	Steroids	Tannins	Phenols	Glycosides
TVFHex-	-	-	-	-	-	-	+
TVFDichl-	+	-	+	+	-	+	+
TVFAcet-	+	-	-	+	+	+	+
TVFMeth-	+	+	+	+	+	+	+
TVGHex-	+	-	+	-	-	-	-
TVGDichl-	+	-	+	+	-	-	+
TVGAcet-	-	-	-	+	+	-	+

TVGMeth-	+	+	-	-	+	+	+
RCFbHex-	+	+	+	+	+	+	+
RCFbDichl-	+	-	+	+	-	+	-
RCFbAcet-	+	-	+	+	-	-	+
RCFbMeth-	+	+	-	-	+	+	+
RCFrHex-	+	-	+	+	-	-	-
RCFrDichl-	-	-	+	+	+	+	-
RCFrAcet-	-	-	+	+	-	-	-
RCFrMeth-	+	+	+	+	+	+	+
RCGbHex-	+	-	+	+	-	+	-
RCGbDichl-	+	-	+	-	-	-	-
RCGbAcet	+	-	+	+	-	+	-
RCGbMeth-	+	-	-	-	+	-	-

Legend : (+) : Presence of secondary metabolite ; (-) : Absence of secondary metabolite

Aqueous extracts testing on A. fabae

Application of respective doses of 250; 125 and 62.5 g/l of aqueous extracts from the fresh leaves of *T. vogelii* and *R. communis* of white and of red leaves on 20 *A. fabae* showed that extracts from the fresh leaves of *T. vogelii* were more effective compared with extracts from the fresh leaves of *R. communis* (white and red leaves). The progressive increase in the mortality rate of *A. fabae* sprayed with *T. vogelii* fresh leaf extracts was observed from day 1 to day 5 of application (Fig. 1). In addition, the mortality rate was higher with extracts of fresh *T. vogelii* leaves at the dose of 250 g/l for which all sprayed *A. fabae* were dead at day 4 of application.

Daily mortality rates were calculated on aqueous extracts of RCFb, RCFr and TVF at 250 g/l and 125 g/l having killed at least 50 % of *A. fabae* during the 5 days after application. TVF extracts showed mortality rates of 20±0.14 % and 13±0.18 % of dead *A. fabae* per day at 250 g/l and 125 g/l, respectively. With the dose of 250 g/l, RCFb and RCFr aqueous fresh leaf extracts resulted in respective mortality rate of 15±0.15 % and 16±0.14 %. According to the Student t-test, these averages for the two doses from the three plant species did not differ significantly (p = 0.7).

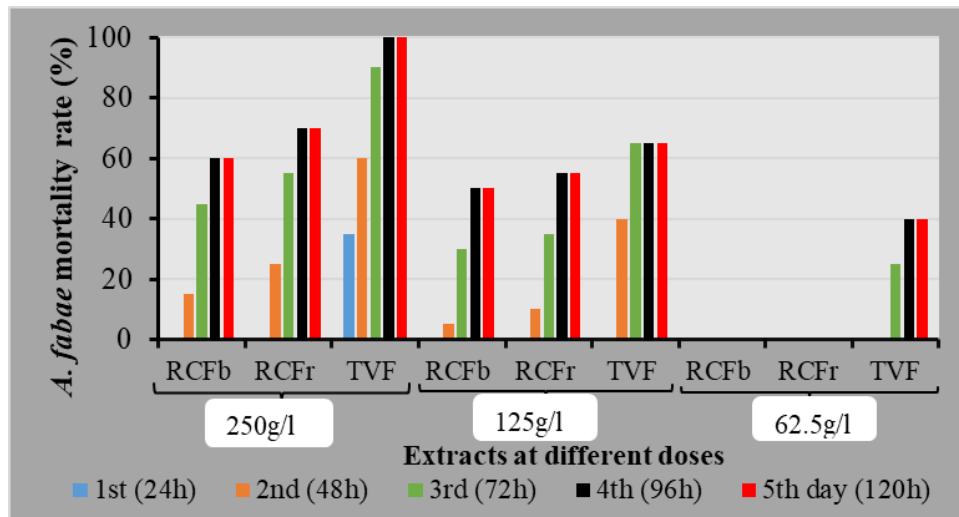


Figure 1 : *A. fabae* mortality as a function of time after application of 250, 125 and 62.5 g/l aqueous extracts of *T. vogelii* (TVF) and *R. communis* white (RCFb) and red (RCFr) leaves

Activity rate of aqueous extracts on *S. frugiperda*

Extracts of fresh *T. vogelii* leaves at the dose of 250 g/l were more effective than extracts of fresh *R. communis* leaves at the same dose. By day 4, almost 65 % of *S. frugiperda* sprayed with fresh *T. vogelii* leaf extract were dead (Fig. 2). Daily mortality rates were used to assess the dose-dependent of each extract. The dose of 250 g/l of TVF extract showed a daily mean mortality rate of 13.4 %. The RCFb and RCFr aqueous fresh leaf extracts, at the same dose, showed a daily mean mortality of $11.2 \pm 0.15\%$ and $11.2 \pm 0.11\%$; respectively. The Student t-test showed that these two averages were not significantly different ($p = 1$).

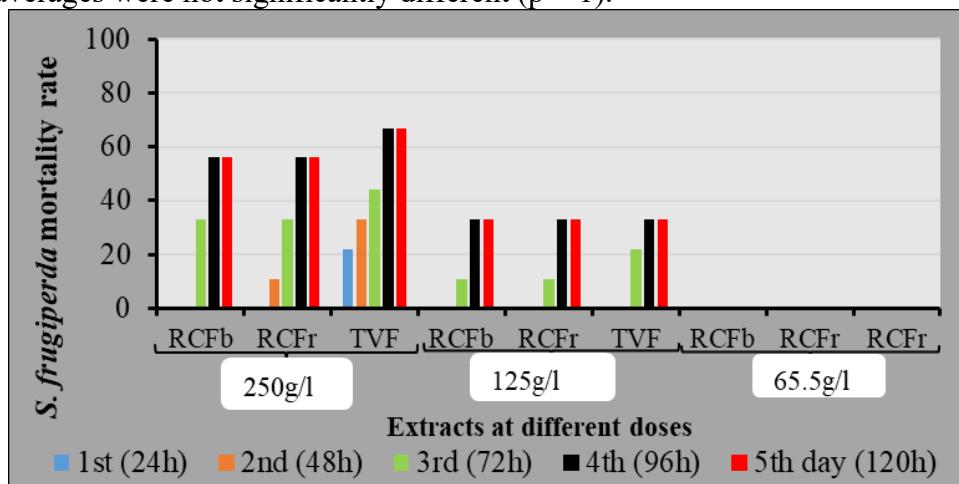


Figure 2: *S. frugiperda* mortality as a function of time after application of 250, 125 and 62.5 g/l extracts of *T. vogelii* (TVF) and *R. communis* white (RCFb) and red (RCFr) leaves.

LD₅₀ of aqueous extracts of *T. vogelii* on *A. fabae*

After 5 days, the *A. fabae* mortality rate was 100% for the 250 g/l aqueous extract of *T. vogelii* leaves. The required dose to kill 50% of *A. fabae* using this aqueous extract was found to be 114 g/l (Table 3).

Table 3: Parameters for determining the lethal dose of aqueous extract of *T. vogelii* leaves

Extract	Doses (g/l)	N	n	%	a	b	(a×b)
TVFAq-	62.5	20	8	40	62.5	10.5	656
	125	20	13	65	125	16.5	2063
	250	20	20	100			
$\Sigma (a \times b)$							2719
LD_{50}							114 g/l

$LD_{50} = LD_{100} - \Sigma (a \times b) / N$, where a: difference between two successive doses; b: half-sum of dead individuals between two successive doses; N: total number of individuals; n: average number of dead individuals.

Solvent extraction of secondary metabolites

According to the obtained results, the secondary metabolites extraction yield depends on the type of organic extract used, organs and the mass of the powder taken as sample. With 100 g of *T. vogelii* and *R. communis* white leaf powder macerated with n-hexane, an extract yield of approximately 3.82 % and 3.2 % were obtained respectively (Table 4).

Table 4: Extraction yields of secondary metabolites from leaves and seeds of *T. vogelii* and *R. communis* white and red leaves

Species	Organs	Mass of powder (g)	Extraction solvents	Mass of extract (g)	Yield (%)
<i>T. vogelii</i>	Leaves	100	n-hexane	3.82	3.82
		96.18	dichloromethane	2.38	2.47
		93.8	ethyl acetate	3.12	3.32
		90.68	methanol	8.88	9.79
	Seeds	42	n-hexane	5.42	12.9
		36.58	dichloromethane	2.22	6.06
		34.36	ethyl acetate	0.79	2.29
		33.57	methanol	1.71	5.09
<i>R. communis</i>	White leaves	100	n-hexane	3.2	3.2
		96.8	dichlorométhane	3.41	3.52
		93.39	ethyl acetate	4.37	4.68
		89.02	methanol	2.64	2.96
	Red leaves	100	n-hexane	2.47	2.47
		97.53	dichloromethane	4.98	5.1
		92.55	ethyl acetate	4.22	4.55
		88.33	methanol	4.55	5.15
	Seeds	150	n-hexane	20.31	13.54
		129.69	dichloromethane	21.9	16.88
		107.79	ethyl acetate	18.4	17.07
		89.39	methanol	4.22	4.72

A. obtectus and S. zeamais mortality for extracts at 4 mg/ml

The 4 mg/ml extracts were the most effective on *A. obtectus* and *S. zeamais*. After 10 days of application, a 100 % mortality rate was observed for *A. obtectus* treated with the hexanic extracts of the leaves of *R. communis* with white and red leaves and the seeds of *R. communis* with white leaves as well. In addition, the dichloromethane and methanol extracts of white leaf seeds and red leaves of *R. communis* (Fig. 3a) achieved a mortality rate of 80 % on *A. obtectus*. A similar mortality rate (Fig. 3b) was observed for *S. zeamais* when the hexanic and ethyl acetate extracts of *R. communis* white leaf seeds were applied 10 days.

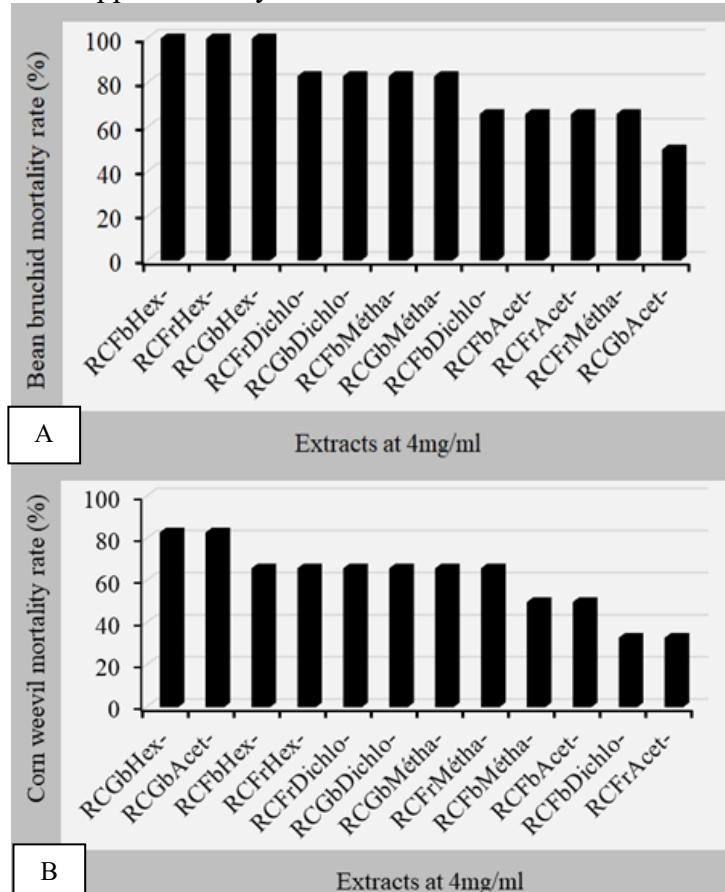


Figure 3: Mean mortality rate of *A. obtectus* (a) and *S. zeamais* (b) by the 4mg/ml extracts of the leaves and seeds of *R. communis* white and red leaves over 10 days. RCFbHex- and RCFrHex- represent Hexane extracts from leaves of *R. communis* white and red respectively, RCGbHex- : acronym of seeds of *R. communis* white ; RCFbDichlo-, RCFrDichlo- and RCGbDichlo-represent Dichloromethane extracts from white and red leaves and seeds of *R. communis* respectively; RCFbMetha-, RCFrMetha- and RCGbMetha- : Methanolic extracts of white, red leaves and seeds of *R. communis* respectively; RCFbAcet-, RCFrAcet- and RCGbAcet-: Ethyl acetate extract of white and red leaves and seeds of *R. communis* respectively

Treatment of *A. obtectus* and *S. zeamais* with 4 mg/ml extracts of the leaves and seeds of *T. vogelli* showed that the hexane extract of the seeds and leaves and the ethyl acetate extract of the seeds were more effective against *A. obtectus* with a 100 % mortality rate (Fig. 4a). In addition, the hexanic extract of *T. vogelii* seeds was also effective against the *S. zeamais* with a mortality rate of 100 % (Fig. 4b).

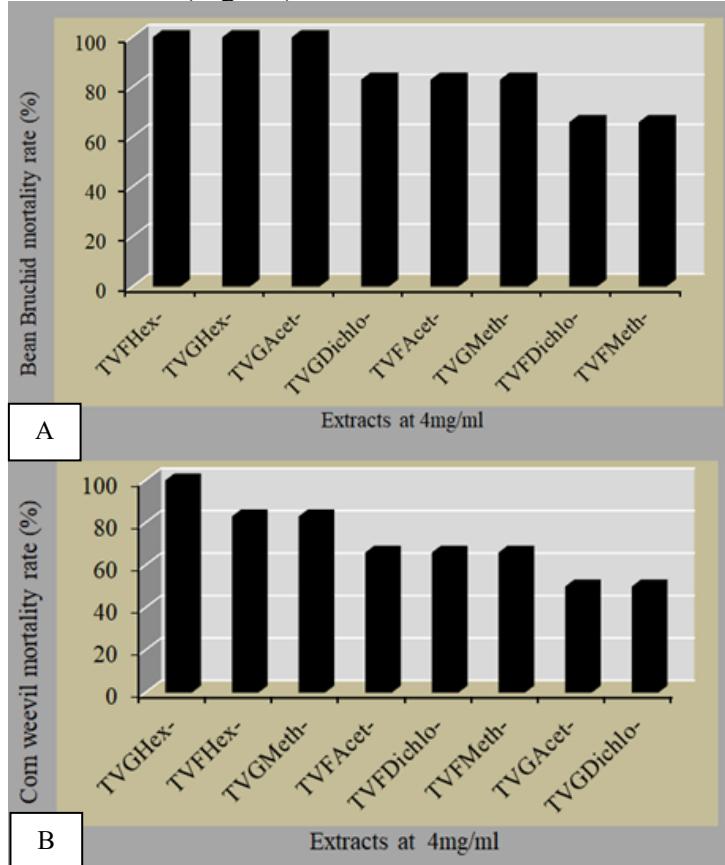


Figure 4: Mean mortality of *A. obtectus* (a) and *S. zeamais* (b) by 4 mg/ml extracts of *T. vogelli* leaves and seeds over 10 days. Hexane extracts of *T. vogelli* leaves (TVFHex-) and seeds (TVGHex-); Ethyl acetate extract of *T. vogelli* seeds (TVGAcet-) and leaves (TVFAcet-); Dichloromethane extracts from seeds (TVGDichlo-) and leaves (TVFDichlo-) of *T. vogelli*; Methanol extracts from seeds (TVGMeth-) and leaves (TVFMeth-) of *T. vogelli*.

Daily mortality rate of *A. obtectus* and *S. zeamais* due to extracts treatment

Daily mean mortality rates of *A. obtectus* and *S. zeamais* were only calculated for extracts of *R. communis* and *T. vogelli* which killed at least 50 % of individuals in 10 days (Fig. 4).

Daily mortality rate of *A. obtectus*

Hexane extracts of *T.vogelii* seeds and leaves at 4 mg/ml showed a daily mean mortality rate of 10 % of *A. obtectus* treated for 10 days with the same extract (Fig. 5). The results obtained from Analysis of Variance showed that the daily mean mortality rates differ according to extract, but no significant difference was observed ($p > 5\%$). The comparison of the daily mean of mortality rates for *A. obtectus* between different extracts did not show any significant difference for the same plant species and for the same extraction solvent. In all cases, the p -value was greater than 5 %. Extracts of RCFbHex (9.4±0.12) and RCFrHex- (9.3±0.13) at 4 mg/ml with $p = 0.9$ and TVGHex- (10.04±0.16) and TVFHex- (10.03±0.14) at 4mg/ml with $p = 1$, showed higher average mortality rates for *A. obtectus*.

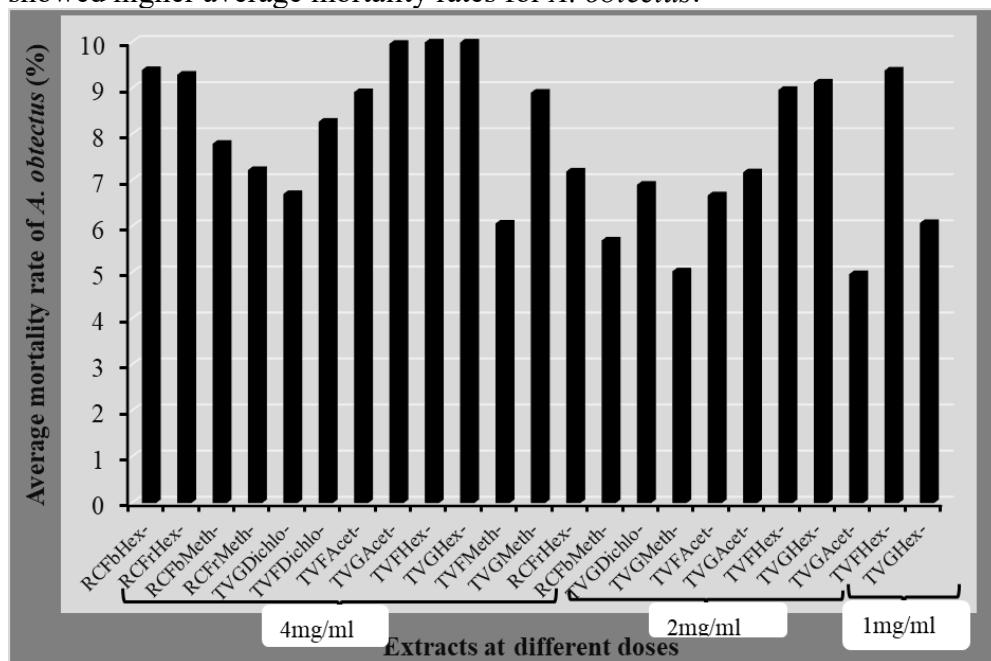


Figure 5: Daily mean mortality rate using leaf and seed extracts of *R. communis* and *T. vogelii*, which decimated at least 50% of *A. obtectus*

Daily mortality rate of *S. zeamais*

It was observed that only TVGHex extract at 4 mg/ml was able to achieve a daily mortality rate of 10% of *S. zeamais* after 10 days of application (Fig. 6). Therefore, for the other extracts, a dose higher than 4 mg/ml would be required to obtain a daily mortality rate of 10% of *S. zeamais*.

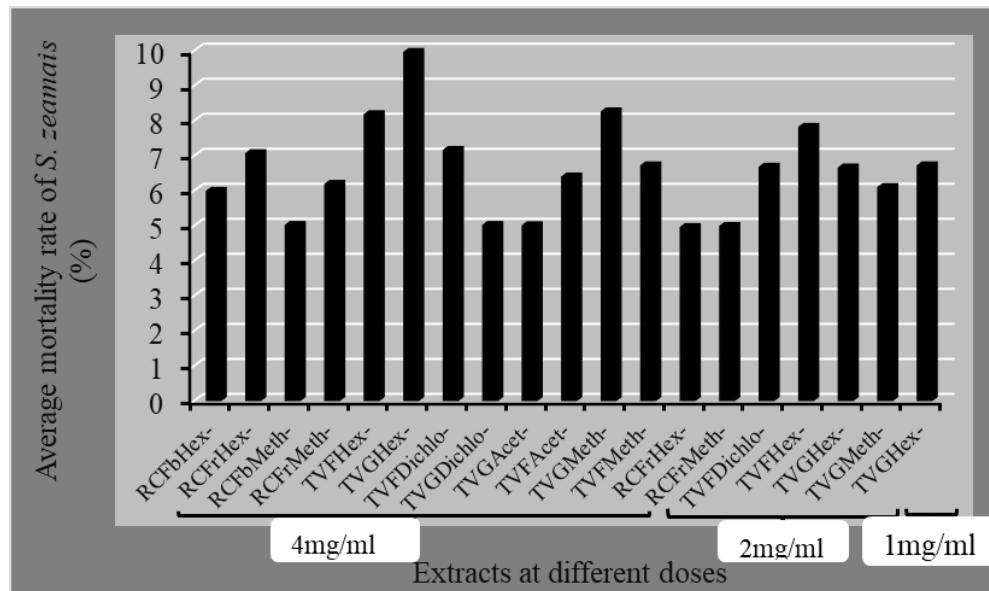


Figure 6: Daily mean mortality using leaf and seed extracts of *R. communis* and *T. vogelii*, which decimated at least 50% of *S. Zeamais*

LD₅₀ of hexane extracts of *R. communis* on *A. obtectus*

The LD₅₀ determined for each hexane extract from the leaves and seeds of *R. communis* white and red leaf on *A. obtectus* are summarized in Table 5. The results showed that the hexane extracts from white and red leaves of *R. communis* could kill 50 % of *A. obtectus* at a dose of 2.16 mg/ml and 1.83 mg/ml; respectively. On the other hand, the hexane extract of *R. communis* white leaf seeds could kill 50 % of *A. obtectus* at a dose of 1.5 mg/ml. This difference in dose shows that the seeds of *R. communis* would be highly toxic compared with the leaf extract. Moreover, the red leaves of *R. communis* seemed to be more effective against *A. obtectus* than the white leaves.

Table 5: Determination of the LD₅₀ of hexane extracts from the leaves and seeds of *R. communis* with white and red leaves on *A. obtectus*

Type of extracts	Dose (mg/ml)	N	n	%	a	b	(a*b)
RCFbHex-	1		6	1	17	1	2
	2		6	3	50	2	4.5
	4		6	6	100		
$\sum (a \times b)$							11
$\frac{LD_{50}}{RCFrHex-}$							2.16mg/ml
RCFrHex-	1		6	2	33	1	3
	2		6	4	67	2	5
	4		6	6	100		10

$\sum (a \times b)$		13
$\frac{\text{LD}_{50}}{\text{RCGbHex-}}$		1.83 mg/ml
1	6 2 33 1 3.5 3.5	
2	6 5 83 2 5.5 11	
4	6 6 100	
$\sum (a \times b)$		14.5
LD_{50}		1.58 mg/ml

$\text{LD}_{50} = \text{LD}_{100} - \sum (a \times b)/N$; where a: difference between two successive doses; b: half the sum of the individuals who died between two successive doses; N: total number of individuals in a Petri dish; n: average number of dead individuals in a Petri dish.

LD₅₀ of hexane and ethyl acetate extracts of leaves and seeds of *T. vogelii* on *A. obtectus*

The hexane extracts of *T. vogelii* leaves at the doses of 1; 2 and 4 mg/ml and that of *T. vogelii* seeds at 4 mg/ml showed a 100% mortality rate on *A. obtectus* after 10 days of treatment (Table 6). The ethyl acetate extract of *T. vogelii* seeds also showed similar performance on *A. obtectus* over the 10-day of treatment. The LD₅₀ determined for each hexane extract and for ethyl acetate extracts of *T. vogelii* seeds on *A. obtectus* are also given in Table 6. The results show that the hexane extracts of *T. vogelii* leaves and seeds can kill 50 % of *A. obtectus* at doses of 1 mg/ml and 1.33 mg/ml; respectively. However, the ethyl acetate extract of *T. vogelii* seeds killed 50 % of *A. obtectus* at the dose of 1.75 mg/ml. These results illustrate that n-hexane and ethyl acetate would be good solvents to extract secondary metabolites from the leaves and seeds of *T. vogelii*. It can be seen that hexane extracts seemed to be more effective than ethyl acetate ones.

Table 6: Determination of the LD₅₀ of hexane and ethyl acetate extracts of *T. vogelii* leaves and seeds on *A. obtectus*

Type of extracts	Dose (mg/ml)	N	n	%	a	b	$(a \times b)$
TVFHex-	1	6	6	100	1	6	6
	2	6	6	100	2	6	12
		6	6	100			
$\sum (a \times b)$							18
LD_{50}							1 mg/ml
TVGHex-	1	6	4	67	1	4.5	4.5
	2	6	5	83	2	5.5	11.5

	4	6	6	100			
$\sum (a \times b)$					16		
LD ₅₀						1.33 mg/ml	
TVGAcét--	1	6	3	50	1	3.5	3.5
	2	6	4	67	2	5	10
	4	6	6	100			
$\sum (a \times b)$					13.5		
LD ₅₀						1.75 mg/ml	

LD₅₀ = LD₁₀₀ - $\Sigma (a \times b) / N$; where a: difference between two successive doses; b: half the sum of the individuals who died between two successive doses; N: total number of individuals in a Petri dish; n: average number of dead individuals in a Petri dish.

LD₅₀ of n-hexane extracts of *T. vogelii* seeds on *S. zeamais*

For all the toxicity tests on *S. zeamais* using the different extracts, only the 4 mg/ml hexane extract of *T. vogelii* seeds killed 100 % of the individuals in 10 days. Thus, determination of the LD₅₀ of the hexane extracts for the *S. zeamais* revealed a dose of 1.66 mg/ml (Table 7).

Table 7: LD₅₀ Calculation for hexane extracts from *T. vogelii* seeds on *S. zeamais*

Type of extract	Dose (mg/ml)	N	n	%	a	b	(a × b)	
TVGHex-	1		6	4	67	1	4	4
	2		6	4	67	2	5	10
	4		6	6	100			
$\Sigma (a \times b)$						14		
LD ₅₀							1.66 mg/ml	

LD₅₀ = LD₁₀₀ - $\Sigma (a \times b) / N$; where a: difference between two successive doses; b: half the sum of the individuals who died between two successive doses; N: total number of individuals in a Petri dish; n: average number of dead individuals in a Petri dish.

Discussion

Based on the results of phytochemical screening of organic extracts, all of the seven secondary metabolite clusters (alkaloids, saponosides, flavonoids, steroids, tannins, phenols and glycosides) were only present in the methanol extracts of the leaves of *T. vogelii* (TVF), red-leaved *R. communis* (RCFr) and the hexane extract of the leaves of white-leaved *R. communis* (RCFb). It should be noted that the organic extraction solvents, methanol and n-hexane, were both able to extract large quantities of the

secondary metabolites present in different parts of the plants. The content of alkaloids, saponosides, flavonoids, steroids, tannins, phenols and glycosides varied depending on the species and organ of the plant, as well as the extraction solvent used. According to Stoll (2002), the plant parts with insecticidal properties are the leaves and roots, with higher levels of secondary metabolites in the leaves, which vary from plant to plant. Indeed, the organic seeds extracts of *T. vogelii* (TVG) and *R. communis* (RCGb) obtained with n-hexane, dichloromethane, ethyl acetate and methanol did not contain all the seven secondary metabolite groups screened. The phytochemical screening of aqueous extracts from fresh leaves of both *T. vogelii* and *R. communis* revealed the presence of glycosides, flavonoids, tannins and phenols. And, in addition to those four secondary metabolites, the aqueous extract of *T. vogelii* leaves (TVF) showed also the presence of saponosids.

The efficacy of aqueous extracts of fresh *T. vogelii* leaves compared with aqueous extracts of fresh *R. communis* leaves on *S. frugiperda* and *A. fabae* could be linked to the presence of saponosides in *T. vogelii* leaves. This is in agreement with the results reported by Stoll (2002), where he mentioned that the leaves and roots of *T. vogelii* have insecticidal properties. In addition, Anjarwalla *et al.* (2016) showed that these plants have toxic properties against insect pests of crops and harvests. (Stoll, 2002) also reported that the leaves and the seeds of *T. vogelii* were effective insecticides against aphids and insects that perforate the stalk of maize, and that the content of secondary metabolites was higher in the leaves. This author reported also the presence of flavonoids in *T. vogelii* leaves with marked effects on insect development and behaviour. It is also shown that flavonoids act by disrupting the insect's natural motricity (Regnault-Roger *et al.*, 2004). The unpleasant taste of flavonoids repels certain insects and plays a role in plant protection (Macheix *et al.*, 2005). The presence or absence of a secondary metabolite in the different extracts from *T. vogelii* and *R. communis* would be responsible of the efficacy rate of each extract on *A. fabae* and *S. frugiperda*, as well as on *A. obtectus* and *S. zeamais*. The toxicity of the aqueous extracts on *S. frugiperda* and *A. fabae* and that of the organic extracts on *A. obtectus* and *S. zeamais* also varied according to the plant species, the organ and the dose used.

The results of this study showed that aqueous extracts from leaves of *T. vogelii* and those from white and red leaves of *R. communis* don't have the same toxic effects on both *A. fabae* and *S. frugiperda*. In fact, the mortality rate of *A. fabae* (100%) and *S. frugiperda* (65%) is higher, 4 days after the treatment, for aqueous extracts of *T. vogelii* leaves; which could be linked to the presence of saponosides in this part of the plant. According to Bruneton (2016) and Krief (2003), saponosides play a role in the plant's defence

against pathogens and microbial attack and are toxic to animals. However, aqueous extracts of leaves of *R. communis* are also somehow effective on *A. fabae* and *S. frugiperda* larvae with around 60% mortality rate. According to Kodjo *et al.* (2011), aqueous and organic extracts of *R. communis* have a strong larvicidal effect. A reduction in second-stage root-knot nematode larvae has also been reported on tomato and banana crops with application of *R. communis* (Ferji *et al.*, 2006, 2013). Ghnimi *et al.* (2014) also demonstrated that aqueous extracts of castor bean leaves and seeds, which contained phenolic compounds, flavonoids and tanins, have larvicidal activity against *Culex pipiens* L., with 100 % mortality after 24 hours of exposure. The toxicity of *R. communis* extracts is thought to be due to ricin, a major bioactive compound of this plant species, the highest concentration of which is found in the seeds. Windholz (1983) postulated that ricin is one of the most toxic natural poisons.

Organic extracts from the seeds and leaves of *T. vogelii* showed good mortality rate on *A. obtectus* and *S. zeamais* in comparison to those of *R. communis*. The difference in the efficiency of extracts from *R. communis* and *T. vogelii* on *A. obtectus* and *S. zeamais* is consistent with their phytochemical screening which revealed differences in secondary metabolites from leaves and seeds of the two plant species. The effectiveness of hexane extracts compared with those using dichloromethane, ethyl acetate and methanol would be linked to the volatile properties of n-hexane, which make it able to extract the majority of secondary metabolites in solution samples. According to Derradji-Heffaf (2013), extracts obtained with volatile organic solvents are more complete, since they contain not only volatile compounds such as terpenes, but also other constituents such as triglycerides, waxes and lipid-based colourants). The difference in efficacy could be attributed to the difference in secondary metabolites present in those extracts and to their concentration.

Higher doses of organic extracts from the leaves and seeds of *R. communis* and *T. vogelii* were effective against *S. zeamais* and *A. obtectus*, but at different levels. The hexane extract of leaves and seeds of *T. vogelii* gave a mortality rate of 100% for the two insects tested, *S. zeamais* and *A. obtectus*; when this mortality rate was also observed for both the leaf hexane extract of *T. vogelii* and that of its seeds. Konna *et al.* (2007) also showed in their study that hexane extract of *T. vogelii* was able to protect stored maize against infestation of *S. zeamais*. It was also observed that the hexanic extracts of the leaves and seeds of *R. communis* gave a mortality rate of 100% for *A. obtectus*; when this mortality rate is given by the hexanic and acetate extracts of its seeds on *S. zeamais*. The use of *T. vogelii*, in the control of *S. zeamais* infestation, is also reported by (Kanana & Muniengi, 2018) using leaf powder in maize seed conservation. Moreover, *T. vogelii* crude

leaf extract was evaluated as a natural acaricide to control ticks on naturally infested traditionally reared cattle by Siame *et al.* (2019). Kamugire and Nshutiyayesu (2020) also reported on the use of *T. vogelii* leaf powder for *A. obtectus* control. According to Bigi *et al.* (2004), ricin is one of the secondary metabolites from *R. communis* that has a very significant insecticidal effect. It was also reported that ricin has a very significant effect on the larvae of *S. frugiperda* (Lepidoptera) (Ramos-López *et al.*, 2010). It was also observed that seeds extracts from *R. communis* and *T. Vogelii* were more effective than their leaves extracts. Ramos-López *et al.* (2010) showed that seed extracts had better insecticidal activity than leaf extracts.

Conclusions

The leaves and the seeds of *R. communis* and *T. vogelii* contain secondary metabolites that are effective against the crop pests studied. However, these secondary metabolites differ from one plant to another and depend on the plant organ. Their aqueous extracts applied to *A. fabae* and *S. frugiperda*, as well as their organic extracts applied to *A. obtectus* and *S. zeamais*, showed different levels of toxicity depending on the dose and solvent used. The highest mortality rates for *A. fabae* and *S. frugiperda* with aqueous extracts were observed after 5 days of treatment. The maximum number of *A. obtectus* and *S. zeamais* killed was obtained after 10 days of treatment with organic extracts. Aqueous extracts of TVF were more effective than aqueous extracts of RCF on *S. frugiperda* and *A. fabae*. The various treatment tests revealed that n-hexane extracts were more effective than dichloromethane, ethyl acetate and methanol extracts. The n-hexane solvent proved to be a good organic solvent for extracting secondary metabolites from the leaves and seeds of *R. communis* and *T. vogelii*, which have a higher toxic effect on the tested insects

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Author's Contribution

Liberata NIZIGIYIMANA : conceptualisation, methodology, investigation, writing-review and editing, project administration ; **Alexis NGENDAKURIYO** : Collecting plant samples, investigation, formal analysis, validation, data curation and writing original draft preparation ; **Jérémie NGEZAHAYO** : Conceptualisation, investigation, Writing - Review; **Déogratias NDUWARUGIRA** : Resources (insect rearing protocol), Writing - Review ; **Aloys KATIHABWA** : Writing - Review; **Manassé NIHORIMBERE** : conceptualisation, Writing - Review

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