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Evaluation of the insecticidal activity of two local plants aqueous extracts (*Azadirachta indica* (Meliaceae), *Cassia siamea* (Cesalpiniaceae)) on termites during the growth of Rice Nerica 1

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ABSTRACT:

This survey was conducted on an experimental parcel of the Agronomic High School of the National Polytechnic Institute Félix HOUPHOUET- BOIGNY (ESA-INPHB) of Yamoussoukro (Côte d'Ivoire). It aims to evaluate the insecticidal effect of 2 local plants aqueous extracts on termites (*Azadirachta indica* and *Cassia siamea*) dosed at 131.25 g powder/ L compared to a chemical pesticide (Chlorpyrifos ethyl) dosed at 480 g / L. A total of 11 termite species were collected from the plots. The fungus-growers with 6 species are the most dominant group. Four soil-feeder species and one wood-boring species were also collected. Only fungus-growers are present in the areas treated with plant extracts and chlorpyrifos ethyl. It was observed a high abundance of termites in heading stage of the rice growth. In maturity stage, ie 4 months after sowing the efficiency of chlorpyrifos ethyl decreased as plant aqueous extracts remain effective. Among the two tested plants, *Azadirachta indica* seems to be the most effective compared to *Cassia siamea* against termites. Chlorpyrifos ethyl could be used as a cure for its swift action on termites while *Azadirachta indica* could be used preventively and for crops whose destructive action is long term.

Keywords:

Rice Nerica, *Cassia siamea*, *Azadirachta indica*, Chlorpyrifos ethyl, Termites.

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INTRODUCTION

Generally in West Africa and in Côte d'Ivoire particularly, rice is one of the most eaten cereals, by people. For several years, rice farmers are confronted to pest problems, and biotic and abiotic stresses (Silvie *et al*., 2012). The variety Nerica was created to increase rice yield, but also to reduce poverty in the world, especially in Africa. Despite efforts, this variety is subject to numerous pest attacks including termites.

Termites are one of the major biotic components of tropical ecosystems where they are, with earthworms and ants, true ecosystem engineers (Lavelle *et al.*, 1997; Dangerfield *et al.*, 1998). According Krishna *et al.* (2013), about 3106 species have been described to date. Among these, nearly 10% are recognized as harmful to living plants and buildings (Logan *et al.*, 1990). Surveys realized on the damage caused by termites are exactly focused on attacks on rice, maize (Johnson *et al.*, 1978; Ouyed, 1990), sugarcane (Mora *et al*., 1996) oil palm crops (Han *et al.*, 1998).

In Côte d'Ivoire, work on termite damage concerned yam (Foua- Bi, 1983), oil palm (Han *et al*., 1998), rubber (Kudu *et al.*, 2004; Tahiri and Mangué, 2007), rice and maize crops (Akpesse *et al*., 2008) and recently the cocoa (Tra-Bi, 2013) and mango crops (Coulibaly *et al.*, 2014). The damage and cost of repairs of the damage were evaluated at over EUR 22 billion each year (Fuchs *et al.*, 2004). Moreover, the control of crops pest termites focuses on the use of chemical insecticides by treating the soil with termiticides. However, intensive and sometimes abusive use of these chemicals is often unsuccessful, but also with non mastered side effects (mammalian toxicity, pest resistance to insecticides and environmental risks) (Su *et al.*, 1998). For all these reasons, it is timely to find reliable alternative methods and environmentally friendly for better management systems and agronomic pests. The use of insecticidal plants extracts seems promising.

In the present survey, the effect of aqueous extracts of two local plants, known for their insecticidal properties will be tested on termites in the crop of rice Nerica 1. The study will contribute to the knowledge of rice pest termites, to evaluate the efficiency of these aqueous extracts on these termites and propose a method of control, with environmental respect, to improve production.

MATERIALS AND METHODS

This study was conducted on a parcel of the Agronomic Higher School of the Félix HOUPHOUET-BOIGNY National Polytechnic Institute (ESA-INPHB) of Yamoussoukro (6° 47 N and 5° 15 W). The soil has a lateritic horizon. The climate is sub-equatorial baouléen kind, warm and humid with 4 seasons. The total height of rainfall in 2010 was 1562.86 mm. The monthly average temperature recorded in the department of Yamoussoukro was 26.4 ° C. The variety Nerica 1 of rice or *Bonfani* (WAB 450-IBP-38-HB) with short cycle of 90 days (semi-maturity), originally from the Côte d'Ivoire (WARDA / BOUAKE) was used. Planting took place in May 2010.

The plant extracts used for tests on the plots are derived from the leaves of *Azadirachta indica* A. Juss., 1830 (Meliaceae) and *Cassia siamea* L., 1783 (Caesalpiniaceae).

The experimental design is a completely randomized Fisher block with 4 treatments (T_0, T_1, T_2 and T_3) and 6 repetitions on a whole surface of 1155 m² (35 m x 33 m). Each elementary plot measuring 20 m^2 (5 m x 4 m). An alley (2m) separates elementary plots. The individual plots include 17 lines of rice plants, spaced 0.25 m. Each line contains 26 seed holes spaced 0.20 m. The sowing period (May 2010) was chosen based on rain starting in this region. The TSBF (Tropical Boil Biology and Fertility) method was used (Anderson and Ingram, 1993). After phytosanitary analysis, the plants are torn to remove the quadrant in the 1 m^2 sub-unit of land randomly selected and defined for this purpose. Three excavation squares (25 cm square and 30 cm deep) were made in each elementary plot. In total, 18 squares excavation per treatment were made. Termites were extracted in successive levels of 0-10 cm; 10-20 cm and 20-30 cm were preserved in alcohol 70%.

The collected individuals are identified under a WILL brand binocular microscope through identification keys of Ahamad (1950); Bouillon and Mathot (1965) and Sands (1959). For the soldierless species, the workers were analyzed using the key of Sands (1972).

Preparation and application of products

Plant aqueous extracts

The leaves of *Azadirachta indica* (T_1) and *Cassia siamea* (T_2) were collected one month before the date of rice sowing. After drying, they were crushed using a blender. 1050 g powder was overthrown in a bucket containing 8 liters of water. A double filtration was made 24 hours after maceration, using a water sieve with a mesh of 1 mm, then with a white tissue type "pergale". One liter of this filtrate was taken to spray each elementary plot of 20 m^2, corresponding to 131.25 g / L of powder.

Reference product

Pyriforce (T_3) dosed at 480 g / L of chlorpyrifos ethyl (organophosphate), was used as reference product. 24 hours after sowing, 80 ml of the insecticide diluted in 2.4 liters of water was used for spraying between rice grooves of elementary plots. Each treatment is replicated 6 times in whole parcel. Control plots (T_0) have not undergone any treatment.

Evaluation of treatments efficacy

The efficiency of treatment was followed throughout the three stages of growth (tillering, heading and maturity). At each stage of growth, three samples per plot were carried out (18 samples / stage / treatment). After collection of termites, their species richness and density (number of termites / m^2) were determined.

Two ecological indices were chosen to describe the stands of each stage of rice's development Nerica 1. Thus, species richness (S) and the index of Shannon-Weaver (H) were determined using a software R (version 2.2.1). The analysis of variance (ANOVA, 2) was performed using SPSS software (version 17.0) followed by comparison of means (Duncan test, $p < 0.05$).

RESULTS

Effect of plant aqueous extracts on the species richness of termites - in tillering stage

Species richness in this stage varies from one treatment to another (Table 1). Seven termite species were collected in untreated plots (T_0) while 5 species in the *Azadirachta indica* treated plots (T_1), 4 species in the *Cassia siamea* treated plots (T_2) and 3 species in the chemical chlorpyrifos ethyl treated plots were collected (T_3). The population of termites in untreated plots is composed of fungus-growers (*Ancistrotermes cavithorax, Microtermes sp*[1], *Odontotermes pauperans* and *Pseudacanthotermes spiniger*) and soil-feeders (*Cubitermes subcrenulatus, Procubitermes sjostedti, Basidentitermes mactus*). In treated plots, only the fungus-growers remain, but the number of species varies according to the treatments. Chlorpyrifos ethyl caused more species loss than the two plant aqueous extracts. The diversity of termites also happens to be reduced in the treated plots.

The Shannon index (H) observed at the tillering stage varies between 0.65 (T_3) and 1.98 (T_0). The index on the untreated plot (H=1.98) is higher than those of the treated plots. The chlorpyrifos ethyl treated plots record the lowest index (H'=0.65) compared to *Azadirachta indica* (H=0.89) and *Cassia siamea* treated plots (H= 0.99) (Table 1).

In heading stage

Species richness in heading stage varies depending on the treatments (Table 1). Eleven species of termites were recorded in untreated plots. However, in the plots treated, 4 species have been collected with

Table I: List of termite species collected in the plots during the different stages of development of rice Nerica 1 (TG = Trophic group: FG = fungus-grower, SF = soil-feeder, WF = wood-feeder

Familly	Sub-/ family	Species	TG	Tillering				Heading				Maturity			
				T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3	T_0	T_1	T_2	T_3
Termitidae	Macro-termitinae	*Ancistrotermes cavithorax* (Silvestri)	FG	*	*	*	*	*	*	*	*	*	*	*	*
		Ancistrotermes guineensis (Silvestri)	FG		*			*	*	*	*	*			*
		Microtermes sp1	FG	*	*	*	*	*	*	*	*	*	*	*	*
		Microtermes sp2	FG		*	*		*		*		*	*	*	*
		Odontotermes pauperans (Holmgren)	FG	*				*				*	*		
		Pseudacanthotermes spiniger (Sjöstedt)	FG	*	*	*	*	*	*	*	*	*	*		*
	Cubiter-mitinae	*Cubitermes subcrenulatus* (Silvestri)	SF	*				*				*			
		Basidentitermes mactus (Sjöstedt)	SF	*				*				*			
		Procubitermes sjöstedti (von rosen)	SF	*				*				*			
	Termi-tinae														
		Pericapritermes urgens (Silvestri)	SF					*							
		Microcerotermes fuscotibialis (Sjöstedt)	WF					*							
		Species richness		7	5	4	3	11	4	5	4	9	5	3	5
		Shannon-Weaver Index		1.98	0.89	0.99	0.65	2.48	0.8	1.49	1.36	2.4	1.21	1.21	1.73

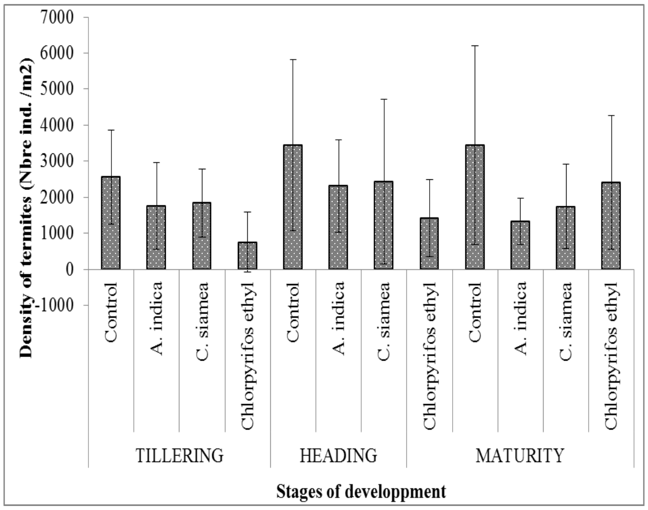

Figure 1: Change in the density of termites based treatments and stages of development

Azadirachta indica (T_1), 5 species with *Cassia siamea* (T_2) and 4 species with chemical chlorpyrifos ethyl (T_3). The population of termites in untreated plots is composed of fungus-growers (*Ancistrotermes cavithorax, Ancistrotermes guineensis, Microtermes sp[1], Microtermes sp[2], Odontotermes pauperans* and *Pseudacanthotermes spiniger*), soil-feeders (*Cubitermes subcrenulatus, Procubitermes sjostedti, Basidentitermes mactus* and *Pericapritermes urgens)* and wood-feeders (*Microcerotermes fuscotibialis*). In treated plots, only the fungus-growers remain with variation in the species composition. There is also a diversity reduction in treated areas.

The Shannon index (H) observed at the heading stage varies between 0.80 (T_1) and 2.48 (T_0). The highest index was observed in the untreated plot (H=2.48). The plot treated with *Azadirachta indica* (T_1) has the lowest value: H=0.80 (Table 1).

In maturity stage

Species richness in maturity stage varies in different plots and depends on the treatments (Table 1). Nine termite species were collected in untreated plots. The populations of termites in these plots are composed of fungus-growers (*Ancistrotermes cavithorax, A. guineensis, Microtermes sp[1], Microtermes sp[2], Odontotermes pauperans and Pseudacanthotermes spiniger*) and soil-feeders (*Cubitermes subcrenulatus, Procubitermes sjostedti* and *Basidentitermes mactus*). Only fungus-growers remain in treated plots, but with variation of the species richness depending on the treatments. *Cassia siamea* extract (T_2) caused more loss of termites than the chemical one (chlorpyrifos ethyl).

Also, the diversity of termites experiencing a reduction in treated plots. The Shannon index (H) observed in maturity stage varies between 1.21 (T_1 and T_2) and 2.40 (T_0). The plots treated with *Azadirachta indica* and *Cassia siamea* record the lowest index (H=1.21) compared to chlorpyrifos ethyl (H=1.73) (Table 1).

Effect of plant aqueous extracts on the density of termites

In tillering stage

The highest density of termites is recorded in the untreated plots (2561.78 ± 1297.31 ind./m^2). Treated plots contain lower densities. The lowest termite density is obtained in plots treated with chlorpyrifos ethyl (751.11 ± 832.76 ind./m^2). The statistical test showed a highly significant difference between treatments (ANOVA, $P < 0.001$) (Figure 1).

In heading stage

The highest density is also recorded in the untreated plots (3446.22 ± 2370.95 ind./m^2). In treated plots, densities are lower. The lowest termite density is recorded in plots treated with chlorpyrifos ethyl (1421.33 ± 1064.002 ind./m^2). The statistical test showed a highly significant difference between treatments (ANOVA, $P < 0.01$) (Figure 1).

In maturity stage

The termite density is higher in the untreated plots (3450.67 ± 2750.67 ind./m^2). Plots treated with aqueous extract of *Azadirachta indica* and *Cassia siamea* have got respectively 1330.67 ± 639.79 ind./m^2 and 1743.11 ± 1167.66 ind./m^2 and have the lowest densities of termites. For that, the plots treated with chlorpyrifos ethyl (2416.89 ± 1853.13 ind./m^2) recorded a higher

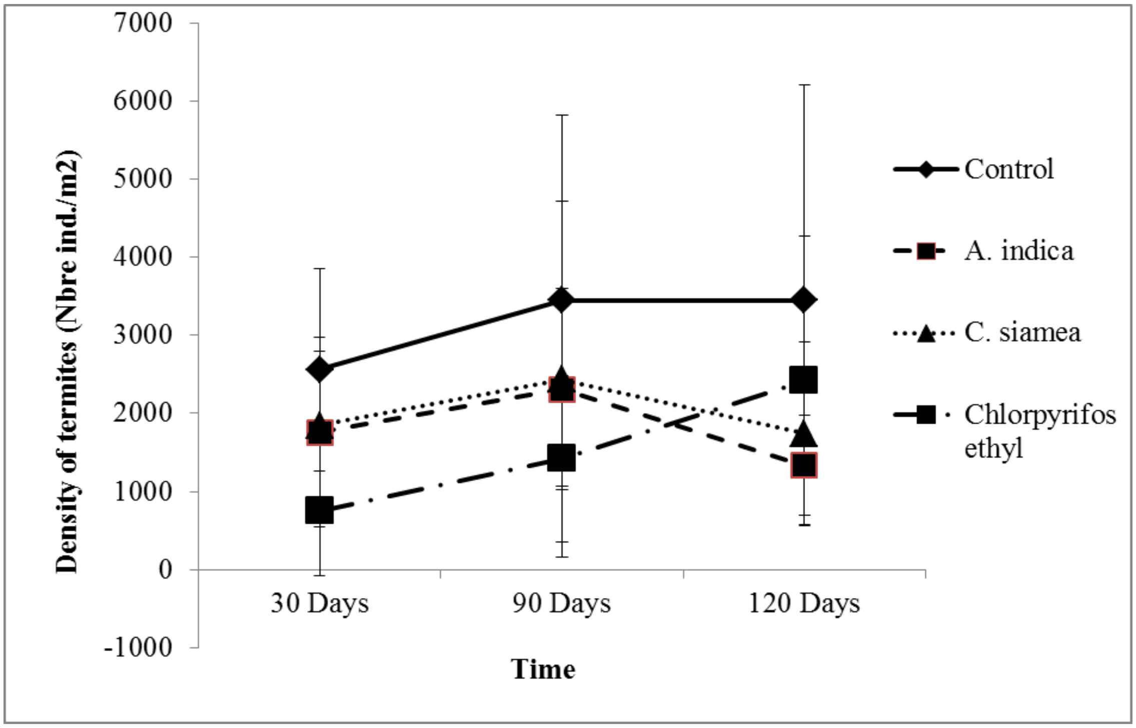

Figure 2: Variation of the insecticidal effect according to the treatment in function of time

density than these two treatments. Statistical test showed a highly significant difference between treatments (ANOVA, $P < 0.01$) (Figure 1).

Evaluation of the insecticidal activity in function of time

The evolution of insecticidal effect of treatments indicates that termites densities recorded in treated plots T_1 (*Azadirachta indica*), T_2 (*Cassia siamea*) and T_3 (chlorpyrifos ethyl) remain significantly lower than the one of the untreated plots (T0) (ANOVA, $P < 0.05$) (Figure 2). The insecticidal effect of treatments on the density of termites, 30 and 90 days after treatment showed no significant difference in the treated plots T_1, T_2 and T_3 (ANOVA, $P > 0.05$). However the efficiency of chlorpyrifos ethyl (T_3) is higher than those using plant aqueous extracts (T_1 and T_2) during the same period. From the 90th to 120th day after treatment, the effect of *Azadirachta indica* (T_1) and *Cassia siamea* (T_2) extracts on the density of termites is significantly higher than the ethyl chlorpyrifos ones (T_3) (ANOVA, $P < 0.05$). The density of termites and the number of species in plants aqueous extracts treatments (T_1 and T_2) are lower than those of chlorpyrifos ethyl (T_3).

DISCUSSION

In total, 11 species of Termitidae grouped into three subfamilies (Macrotermitinae, Cubitermitinae and Termitinae) in the classification of Krishna *et al.* (2013) were collected. They are generally recognized as termite species in cultivated areas (Bodot, 1964; Wood *et al*. 1980; Tra Bi *et al*. (2010); Coulibaly *et al*. (2013) and rice crops, particularly (Akpesse *et al*., 2001). Akpesse *et al*. (2008) have recorded 7 species in rice crops in Booro-Borotou region (North Côte d'Ivoire). This difference is due to the absence of chemical inputs in the cultivation of rice in this survey. Some chemical inputs in the development of crops could prevent colonization of the environment by termites.

The fungus-growers are the trophic group found in all treated areas, particularly the species

Ancistrotermes cavithorax and *Microtermes sp*[1]. This group of termites has always prevailed in the crops, because considered as pests (Mora *et al*., 1996; Han *et al.*, 1998). Their ability to live in the operated areas would be due to their remarkable adaptation, favored by the symbiotic relationship with fungi of the genus *Termitomyces* (Guedegbe *et al*., 2008). This fungus degrades wood fragments which become easier to digest for termites (Matoub, 1993).

Treated plots recorded lower specific richness and densities than untreated plots. This low presence in the treated plots could be explained by the effect of insecticide products used. Several studies have shown that a great anthropogenic activity related to the massive use, often abusive of inputs, affect the trophic structure and species richness of termites (Dibog *et al*., 1999; Jones *et al*., 2003; Eggleton *et al*., 2002; Donovan *et al.*, 2007).

The density of termites varies significantly from a stage of rice development to another. In fact, the density in heading stage is higher than those of the two other stages (tillering and maturity). This could be explained, firstly, by the time between tillering and heading stages, which is estimated at 2 months.

In fact, this term would permit termites to colonize the parcels during a long period before sampling. Furthermore, the heading stage, included in the reproductive phasis, would release a kairomone which attract insects during the formation of reproductive organs of rice. According to Silvie *et al.* (2012), the high attack period of major pests of rice would begin from the end of tillering to the beginning of maturation.

In addition, the termite density varies significantly from a treatment to another, lower in treated plots than in untreated plots due to the insecticidal effect of aqueous extracts of plants (*Azadirachta indica* and *Cassia siamea*) and chemical (chlorpyrifos ethyl). From tillering stage to heading stage, respectively 30 and 90 days after treatment, the density of termites in plots treated with chlorpyrifos ethyl is lower than the one treated with plant extracts. There would be some efficiency of chlorpyrifos ethyl against termites. This product showed high toxicity and rapid action against pests termites in rubber crops (Tran *et al*., 1998; Tahiri *et al.*, 2008; 2011). Although the aqueous extract from *Azadirachta indica* contains phenolic compounds (flavonoids and tannins) and saponins (Wardell, 1987; Okamura, 1993; Tahiri *et al.*, 2011), this extract did not show rapid action on termites 60 days after treatment. Similarly, the aqueous extract of *Cassia siamea*, which contains alkaloids, tannins, saponins, flavonoids and quinones (Ingkaninan *et al*., 2000; Nsonde-ntandou *et al.*, 2005, Morita *et al*., 2007), showed no early action compared to Chlorpyrifos ethyl. The low efficiency of the plant extracts could be explained by the dose used in this survey. The single dose of 131.25 g leaves powder/ L might be low to have a similar action to the chemical. Tahiri *et al.* (2010) demonstrated in laboratory that the most toxic alcoholic extracts of seeds and hexane extract of papaya pulp (Caricaceae), have their low dose optimal actions against termites. However, the efficiency of the extract of Kinkeliba, *Combretum micranthum* (Combretaceae) increases according to the dose used (Tahiri, 2012). The type of solvent used in this survey could have an effect on the low efficacy of extracts during this growth phases of the rice because according Morallo and Tantengo (1986), Teugwa *et al.* (2002), the efficiency of a product depends on the solvent used. The efficiency of used treatments appears to vary along the time. 120 days (4 months) after the treatments application, the effect of aqueous extracts significantly differs and remains higher than the chlorpyrifos ethyl ones. This loss of efficiency could be explained by the degradation of chlorpyrifos ethyl along the time. Tahiri *et al*. (2008) states that the persistence of chlorpyrifos ethyl is about 4 months. In this survey, the chlorpyrifos ethyl was assayed at 480g / L EC liquid formulation was used while Tahiri *et al.* (2008) used chlorpyrifos ethyl dosed at 670 g / L per hectare and with solid formulation or granule. Chlorpyrifos ethyl solid formulation or granule has a slow distribution in soil compared to a liquid formulation, which allowed the product to have a long persistence in the survey of Tahiri *et al.* (2008). Chlorpyrifos ethyl liquid changing fast in the ground and under the effect of temperature evaporates as significant vapor beyond 20 ° C (Walia *et al.*, 1988; Tahiri *et al.*, 2008), which has probably result in the loss of efficiency on termites. According these authors, higher the dose of chlorpyrifos ethyl is, higher it is toxic and so does the efficiency on termites. Certainly all of these conditions have influenced the efficiency of ethyl chlorpyrifos in our survey.

However, the decline in termite density in plant extracts treated areas could be related to two factors. First, the chemical composition of the two extracts in phenolic compounds (flavonoids and tannins), saponins and saponin, quinones, terpenoid and alkaloids whose insecticidal effects were mentioned by several authors (Wardell, 1987; Okamura, 1993; Nozzolillo *et al.* 1997; Bouchelta *et al.*, 2005). The second factor could be linked to the system of trophallaxis. The aqueous extract of *Azadirachta indica* is able to be transmited from treated individuals in the colony during social tasks, contact and licking (Tahiri *et al.*, 2011).

CONCLUSION

After this survey, it is revealed that termites species richness varies from a stage to another, as their density. Under the conditions of this study, chlorpyrifos ethyl assayed at 480 g / L loses its effectiveness after 4 months treatment. Efficacy of *Azadirachta indica* and *Cassia siamea* as pesticides was evaluated. The persistence of the two plant aqueous extracts lasts longer than 4 months. These results seem promising in the control of pests termites. A thorough study should be conducted to know the mode of action of plant extracts on termites

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