Pest Technology ©2008 Global Science Books

Citronella Oil Inhibits Cotton Ramulosis in Controlled Conditions

Waléria Guerreiro Lima[1] • Roseane Cavalcanti Santos[2] • Cláudio Augusto Gomes da Câmara[3] • Marcos Paz Saraiva Câmara[1] • Péricles de Albuquerque Melo Filho[1*]

[1] Agronomy Department, Phytopathology Área, Universidade Federal Rural de Pernambuco (UFRPE), R. Don Manoel de Medeiro, s/n, Dois Irmãos, Recife-PE. CEP. 52171-900, Brazil

[2] Biotechnology Area, Empresa Brasileira de Pesquisa Agropecuária, Embrapa Algodão, CP 174, CEP. 58107-720, Campina Grande, PB, Brazil

[3] Chemical Department, UFRPE, Brazil

Corresponding author: * **pericles@depa.ufrpe.br**

ABSTRACT

Ramulosis is a serious disease affecting cotton crops in Brazil. Control is often based on the use of chemical fungicides. Aiming to study an alternative form of control, we assessed the curative and preventive effects of citronella (*Cymbopogon citratus* (DC) Stapf.) oil in cotton plants based on epidemiological components, in controlled conditions. Trials were conducted with the susceptible cv. 'BRS 8H' and *Collethotrichum gossypii* var. *cephalosporioides* (strain Cgc 287) at 1.0×10^6 conidia mL^{-1}. Citronella oil was used at a concentration of 2000 SI. Assessment of the incidence and severity of ramulosis was based on Initial Disease Index (IDI), Final Disease Index (FDI), Disease Progress Rate (DPR) and Area Under Disease Progress Curve (AUDPC). In the curative treatment, citronella oil showed a limited effect at the concentration used but in the preventive aspect, the FDI in cotton plants was reduced providing more protection to them against the fungus severity.

Keywords: *Collethotrichum gossypii*, crop protection, epidemiology, Essential oil, fungicide, *Gossypium hirsutum*
Abbreviations: **AUDPC**, Area Under Disease Progress Curve; **DPR**, Disease Progress Rate; **FDI**, Final Disease Index; **IDI**, Initial Disease Index

INTRODUCTION

Ramulosis (*Colletotrichum gossypii* South. var. *cephalosporioides*) is one of the main fungal diseases occurring in cotton crops. Damage occurs to the entire plant, with greater harm caused to young plants, as the terminal shoots may become re-infected and raise the degree of physiological damage stemming from the reduction in vegetative growth (Cia and Salgado 2005). Losses incurred by the disease may reach 80% or more, depending upon the susceptibility of the cultivar, the age of the affected plant and climatic conditions (Freire *et al.* 1997; Suassuna and Coutinho 2007). Productivity, weight of the boll, length and thickness of the fiber and weight of the seeds are the most affected characteristics in cotton crops (Carvalho *et al*. 1984).

The main control method is by sowing of healthy seeds or treatment of seeds with fungicides, as the availability of commercial cultivars with ample resistance to the disease is limited. Regarding the efficiency of chemical fungicides in the control of the disease, the use of such products by farmers is at times performed without criteria or sufficient technical information, causing harm to human health and the environment as well as favoring the emergence of new populations of fungus that are resistant to the product used.

In recent years, the intensification of health concepts among society in an effort to find healthier alternatives in agriculture while preserving the environment has promoted a more ecological form of agriculture management, thereby minimizing the use of synthetic chemical products (Bird *et al*. 1990). Alternative control is understood as the integration of non-polluting measures applied in a preventive fashion with the aim of reducing the intensity of disease while increasing production, productivity and the quality of the agricultural products. Such methods stress the deployment of cultural, mechanical, physical, legislative, biological and genetically resistant tactics aimed at prevention and reduction in the intensity of diseases (Paula Júnior *et al.* 2005).

Among plant compounds, essential oils have the greatest biological application as anti-microbial agents. This is an extension of the very role they exercise in plants, defending them from pathogenic bacteria and fungus. There are a number of reports on the performance of these compounds in the control of phytopathogens, inhibiting the growth and germination of fungus in small concentrations of the vegetal extracts. With cotton, however, such studies are scarce. Considering the economic importance of this crop to Brazil, such studies are relevant. They may reveal alternative forms of control, thereby minimizing production costs (Valarini *et al*. 1994).

The aim of the present study was to assess the curative and preventive effect of citronella (*Cymbopogon citratus* (DC) Stapf) oil in the control of ramulosis in cotton crops based on epidemiological components.

MATERIALS AND METHODS

Planting and inoculation of seedlings

Cleaned and disinfected cotton seeds from susceptible cv. 'BRS 187' ('CNPA 8H'), provided by Embrapa Algodão (Campina Grande, PB, Brazil), were used. The *C. gossypii* var. *cephalosporioides* (Strain Cgc 287) was obtained from cv. 'BRS Aroeira' from Montvidiu, GO, Brazil, with symptoms of the disease. Leaf fragments with symptoms were sterilized with 1.5% sodium hypochlorite, placed in BDA and incubated at 25°C for one week. Later, Koch's postulates were confirmed, with the re-inoculation and reproduction of the symptoms.

The citronella essential oil was provided by the Chemical Department (UFRPE). The aerial parts were submitted to hydrodistillation for 2 h and the oils were collected by a modified methodology.

Cotton seeds were sown in plastic containers (8 L capacity)

Received: 8 November, 2007. Accepted: 8 February, 2008.

Original Research Paper

Table 1 Rates of mycelial growth, germination and sporulation inhibitions of *C. gossypii* var. *cephalosporioides* obtained under different concentrations of essential oils from six different botanical species.

Botanical species	Mycelial growth		Germination		Sporulation	
	SI	IT (%)	SI	IT (%)	SI	IT (%)
Piper marginatum	500	92.5 ± 1.23	2500	41.4 ± 0.96	2500	48.1 ± 0.96
Lippia gracillis	1500	93.1 ± 1.05	2500	72.4 ± 1.01	2000	93.2 ± 1.56
Cymbopogus nardus	1500	94.3 ± 1.32	1000	96.2 ± 1.21	1000	100.0 ± 0.01
Eucalptus citriodora	2500	87.2 ± 0.96	2500	91.7 ± 0.89	2000	98.1 ± 1.22
Malpighia glabra	2500	41.7 ± 2.24	2500	41.7 ± 1.36	2500	59.4 ± 1.58
Hibiscus cannabinus	2500	22.2 ± 0.86	2500	6.1 ± 0.69	2500	5.4 ± 0.86
CV (%)		16.23		10.2		12.4

IT, Inhibition rate; CV, Coefficient of Variation

NOTE: Synthesis of a previous *in vitro* assay carried out in the Mycology Laboratory at the Agronomy Department of UFRPE using six different essential oils at 500, 1000, 1500, 2000 and 2500 SI. Each oil was mixed with 20 ml PDA broth (45-50°C) and laid in a Petri dish. An 8 mm diameter disc containing *C. gossypii* var. *cephalo-sporioides* mycelia in solid PDA broth was cut from the periphery of the growth area and placed top-face down on the centre of the Petri dish and properly incubated. This table simply displays the best results at each oil concentration.

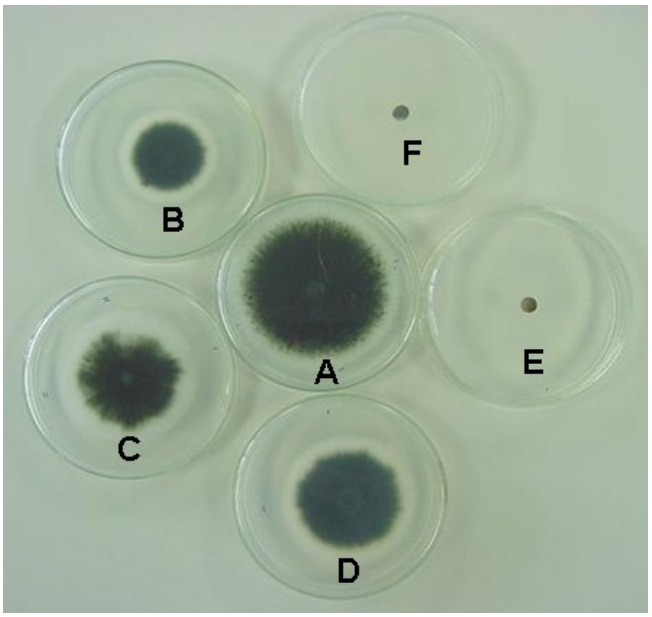

Fig. 1 Mycelia growth of *C. gossypii* var. *cephalosporioides* under different concentrations of citronella oil. A, control; B, 1500 SI; C, 1000 SI; D, 500 SI; E, 2000 SI; F, 2500 SI.

containing previously sterilized and fertilized Latosoil soil, maintaining five plants per pot. The trial was carried out in a greenhouse of the Agronomy Department of the Universidade Federal Rural de Pernambuco (UFRPE), during middle winter, from July to September 2006. Average daily and nightly temperatures and relative humidity of the air during the experiment were 28°C, 19°C and 85%, respectively. At 25 days after planting, when plants exhibited *ca*. four definitive leaves, the treatments were differentiated as to preventive and curative effects of citronella oil for the incidence and severity of ramulosis. The preventive effect of the oil was compared to the preventive effect of the fungicide thiophanate-methyl (Cercobin 500 SC, Iharabras, Sorocaba, SP, Brazil).

The concentration of fungus suspension used in all treatments was 1.0×10^6 conidia mL^{-1}. As for citronella oil, the concentration used was 2000 SI, based on previous trials carried out at the Mycology Laboratory of the UFRPE (**Table 1, Fig. 1**). The following treatments were employed: 1 – Plants inoculated with a conidia suspension (control); 2 – Plants inoculated with the fungus and previously treated with citronella oil (curative treatment); 3 – Plants treated with citronella oil and later inoculated with the fungus (preventive treatment with oil); and 4 – Plants treated with fungicide and later inoculated with the fungus (preventive treatment with fungicide).

Curative treatment was carried out at 28 days following planting, three days after inoculation, when plants exhibited the first symptoms. The plants of the preventive treatment (Treatments 3 and 4) were treated with citronella oil or with a thiophanate-methyl–based fungicide (5 g of the commercial product/L of water, following the manufacturer's recommendations) 25 days after planting. Twenty-four hours later, the plants were inoculated with the conidia suspension and placed in a humidity chamber for 72 hours. An entirely randomized experimental design was used, with five replications.

Incidence and severity of ramulosis

Evaluations were performed at three-day intervals after inoculation, during thirty days. The following epidemiological components were calculated:

a) Initial Disease Index (IDI): The scoring was done at 28 days after emergence, calculated according to McKinney (1923) by the formula: IDI = Sum of grades × 100/ Total number of leaves assessed × maximum disease grade. Scores for the disease severity were graded on a numerical rating scale ranging from 0 to 4;

b) Final Disease Index (FDI): The scoring was done at 55 days after emergence, calculated as described above, to IDI;

c) Disease Progress Rate (DPR), estimated by the *b* parameter from a simple linear regression equation. The linear model was chosen based on the highest determination coefficient of the regression (R^2) for reciprocity between observed and predicted values of the disease incidence as well as the low residual mean square error and absence of undesirable tendencies in the residual plot graph for most curves;

d) Area Under the Disease Progress Curve (AUDPC), calculated by the expression:

$$\sum_{i=1}^{n} [(y_i + y_{i+1})/2]d_{ti}$$

where n = number of assessments, y_i and y_{i+1} = severity values observed in two consecutive evaluations and d_{ti} = interval between evaluations (Shaner and Finney 1977).

The data were transformed into $\sqrt{(x + 0.5)}$ and submitted to analysis of variance. Averages were compared by the Tukey test, at the 5% probability level. The SAEG program (Statistical and Genetic Analysis Systems, Universidade Federal de Viçosa, MG, Brazil, 2003) was used.

RESULTS AND DISCUSSION

In this work, we assessed the curative and preventive effect of citronella (*Cymbopogon citratus*) oil in the control of ramulosis in cotton plants based on epidemiological components. After inoculation of plants in greenhouse, the initial appearance of fungus symptoms occurred three days following inoculation and was characterized by the emergence of necrotic spots on the leaves and petioles, with a predominance of spots on younger leaves. Later, a shortening of the internodes, death of the apical bud and overbudding were observed. No phytotoxic effect of the oil was observed on the plants at the concentration used.

Table 2A displays the epidemiological components of the disease obtained in the curative treatment. Statistically significant differences were observed for to Area Under of Disease Progress Curve (AUDPC) and Disease Progress Curve (DPR), both of which were greater in the control. The Final Disease Index (FDI) was high for both treatments

Table 2 Epidemiological components evaluated in curative (A) and preventive (B) treatments of ramulosis with citronella oil.

A: Curative treatment

Treatment	AUDPC	FDI (%)	DPR (%)
Curative	67.76 ± 1.36 a	98.00 ± 2.73 a	3.00 ± 0.03 a
Control	72.62 ± 2.15 b	100.00 ± 0.01 a	73.00 ± 0.01 b
CV (%)	22.46	20.76	19.57

Area Under the Disease Progress Curve (ABDPC), Final Disease Index (FDI), Disease Progress Rate (DPR). Means with the same letters are not significantly different according to Tukey's test ($P \leq 0.05$). CV, Coefficient of Variation

B: Preventive treatment

Treatment	AUDPC	IP	IDI (%)	FDI (%)	DPR (%)
Preventive	5.98 ± 0.65 a	2.00 ± 0.45 a	1.00 ± 0.09 a	11.00 ± 1.65 a	71 ± 0.05 a
Fungicide	16.64 ± 1.25 b	4.73 ± 0.85 a	8.00 ± 0.45 b	20.00 ± 2.15 b	71 ± 0.36 a
Control	72.62 ± 0.96 c	5.32 ± 1.65b	50.00 ± 1.45 c	100.00 ± 0.01 c	73 ± 0.45 b
CV (%)	26.20	12.21	17.97	26.60	29.89

Area Under the Disease Progress Curve (AUDPC), Incubation Period (IP), Initial Disease Index (IDI), Final Disease Index (FDI), Disease Progress Rate (DPR). Means with the same letters are not significantly different according to Tukey's test ($P \leq 0.05$). CV, Coefficient of Variation.

and these results were favored by the temperature (28 ± 2°C) and humidity (85 ± 5%) conditions, which were favorable to the fungus development. Carvalho *et al*. (1971) studied the physiology of *C. gossypii* var. *cephalosporioides* using strains collected in cotton plants from the Brazilian Cerrado and reported maximum fungal growth at 28°C. According to authors, under 15°C and above 33°C, fungus growth was greatly reduced. In Viçosa, MG (Brazil), Santos *et al*. (1994) found an increase in the incidence of ramulosis under field conditions at 81 days after emergence when relative humidity and minimum temperature reached maximum values of 90% and 18°C, respectively. The paralysis of the disease coincided with the scarcity of rains and with a reduction in temperature to 12.8°C, thereby demonstrating that these climatic variables are important to the development of the disease. Maximum severity of the disease was equal for all treatments at 81 days.

Considering these results and the volatility of the essential oils of the plants, a new application of the product before this 81-day period could theoretically imply a lower FDI than that found in the control treatment. Based on showed data, however, the citronella oil applied for curative purpose had a limited effect at the concentration used (2000 SI) (**Table 2A, Fig. 2**). This may be a consequence of the oil failing to act upon the fungal structures in the interior of the vegetal tissue, thereby contributing toward raising the FDI. The DPR obtained (3%) was very low in comparison to the control (73%), indicating that disease progressed very slowly in the plants treated with citronella oil. According to Parlevliet (1979), a low Disease Progress Rate (DPR) implies a greater time for the disease to reach epidemic levels, thereby characterizing the behavior of the plants as having high horizontal resistance.

It was not possible to assess the incubation period (IP) and initial disease index (IDI) for the curative treatment, as

Fig. 2 Plants inoculated with the fungus and later treated with citronella oil (curative treatment).

Fig. 3 Plants treated with citronella oil and later inoculated with the fungus (preventive treatment).

Fig. 4 Plants treated with fungicide and later inoculated with the fungus (preventive treatment).

the treated plants exhibited similar behavior to the control. In both cases, the symptoms began to appear on the third day following inoculation, soon after removal from the humidity chamber.

Table 1B displays the epidemiological components obtained during the preventive treatment trial. Statistically significant differences were observed between the treatments and the control for IP and DPR. The values obtained for AUDPC, IDI and FDI in the treatment with citronella were lower than those obtained with the fungicide. This means that, despite DPR was statistically similar for both citronella oil and fungicide treatments, the FDI of oil-treated plants was lower than those of fungicide, which exhibited a greater disease severity and, consequently, a greater AUDPC.

Table 3 Average of bud productions from cotton plants submitted to curative and preventive treatment with citronella oil and fungicide.

Treatment	Bud Numbers *	% in relation to control
Preventive-Oil	8.6 ± 0.54 a	87
Preventive-Fungicide	7.8 ± 1.03 a	69
Curative-Oil	4.8 ± 0.65 b	4
Control	4.6 ± 0.55 b	-
CV (%)	11.7	-

*Original data transformed in √(X + 0.5). Means with the same letters are not significantly different according to Tukey's test ($P \leq 0.05$). CV, Coefficient of Variation.

This finding is highly relevant to the alternative control of ramulosis and opens new horizons for further investigations regarding its efficiency and economic advantages under natural field conditions. A detail of plants submitted to preventive treatments are shown in **Figs. 3, 4**.

Total production of floral buds during trial period was also recorded in both curative and preventive treatments. The number of buds in plants submitted to citronella oil in preventive treatment was statistically similar to those fungicide-treated plants ($P \leq 0.05$, **Table 3**), with an average yield increase of 78 % in relation to control. Considering only treatments with citronella oil, the increase in the number of floral buds due to preventive and curative treatment was 87% and just 4%, in comparison to the control, respectively.

The potential of citronella oil for pest control has been largely studied by several researchers, p.ex. in *Tetranychus urticae* (Acari:Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae) (Choi *et al.* 2004), in *Hyadaphis foeniculi* Passerini (Hemiptera: Aphididae) (Abramson *et al.* 2006), in *Culex pipiens quinquefasciatus* (Diptera: Culicidae) (Yang *et al.* 2005), etc. As to its potential for disease control, some tests performed with fungi showed expressive results as seen in Thanaboripat *et al*.'s (2004) study which reported that citronella oil at 0.2% (v/v) inhibited the growth of *Aspergillus flavus* IMI 242684 on PDA for 21 days; Goubran and Holmes (1993) reported fungicidal properties of citronella oil at concentrations of 1900–2000 mg/L (0.19–0.20% v/v) against *Monilia* and *Rhizopus* and Somda *et al*. (2007) evaluated the inhibitory activity of citronella (lemongrass), *Eucalyptus camaldulensis* (eucalyptus) and *Azadirachta indica* (neem) against *Colletotrichum graminicola*, *Phoma sorghina* and *Fusarium moniliforme* in naturally infected sorghum seeds. Authors reported that an aqueous extract of citronella exhibited the best control effect on seed infection by *C. graminicola* and *P. sorghina* and infections by both fungi were significantly reduced by more than 60% when using a 15% concentration and by 100% when using a 30% concentration of citronella. According to the authors, citronella extract did not affect seedling development.

Considering these aspects and the findings found out in this paper regarding the epidemiological components, citronella oil constitutes an interesting alternative in the preventive control of cotton ramulosis. Nowadays, cotton has assumed an important role in the Brazilian agricultural economy, mainly in the Cerrado region (Freire 2007). Due to the high levels of precipitation that occurs in this region, problems with fungal diseases often takes place, especially with ramulosis, that spreads in several areas where cotton is grown. The damage provoked to the cotton field is high, over than 60% in losses (Freire *et al.* 1997). The control is often made by chemical products which enhances significantly the production cost. The further perspective of application of this oil in a integrated disease management strategy could be advantageous, as they are economically viable, environmentally safe and socially acceptable.

Trials in field are in progressing in order to validate these results, along with cost/benefit analysis to estimate the viability of the method for the further use by the growers.

ACKNOWLEDGEMENTS

Grants for this study were gratefully provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). To Mr. Adriano Márcio da Silva for support in conducting experiments.

REFERENCES

Abramson CI, Wanderley PA, Wanderley MJA, Miná AJS, Souza OB (2006) Effect of essential oil from citronella and alfazema on fennel aphids *Hyadaphis foeniculi* Passerini (Hemiptera: Aphididae) and its predator *Cycloneda sanguinea* L. (Coleoptera: Coccinelidae) *American Journal of Environmental Sciences* **3 (1)**, 9-10

Bird GW, Edens T, Drummond F, Groden E (1990) Design of pest management systems for sustainable agriculture. In: Francis CA, Flora CB, King LD (Eds) *Sustainable Agriculture in Temperate Zones,* John Wiley, NY, pp 55-110

Carvalho LP, Carvalho JMF, Lima EF, Cavalcante FB (1971) Influência da concentração de esporos e da patogenicidade de *Colletotrichum gossypii* South. Var. *cephalosporioides* A. S. Costa e avaliação da resistência de cultivares e linhagens de algodoeiro herbáceo à ramulose. *Fitopatologia Brasileira* **6**, 395-402

Carvalho LP, Cavalcante FB, Lima EF, Santos EO (1984) Influência da Ramulose nas características de fibra e produção do algodoeiro. *Fitopatologia Brasileira* **2**, 593-598

Cia E, Salgado CL (2005) Doenças do algodoeiro (*Gossypium* spp.). In: Kimati H, Amorim L, Rezende JAM, Bergmamim Filho A, Camargo LEA (Eds) *Manual de Fitopatologia: Doenças das Plantas Cultivadas* (4rd Edn), Agronômica Ceres, São Paulo, **2**, pp 42-52

Choi W, Lee S, Park S, Ahn Y (2004) Toxicity of plant essential oils to *Tetranychus urticae* (Acari: Tetranychidae) and *Phytoseiulus persimilis* (Acari: Phytoseiidae). Journal of Economical Entomology **97 (2)**, 553-558

Freire EC, Soares JJ, Farias FJC, Arantes EM, Andrade FP, Paro H, Laca-Buendia JP (1997) *Cultura do Algodoeiro no Estado de Mato Grosso*, EMBRAPA-CNPA, Circular Técnica 23, Campina Grande, pp 65

Freire EC (2007) História do algodão no cerrado. In: Freire EC (Ed) *Algodão no Cerrado do Brasil*, Associação Brasileira dos Produtores de Algodão, Brasília, Brazil, pp 21-52

Goubran FH, Holmes RJ (1993) The development of alternative fungicides from essential oils - Rural industries Research and Development Report. Institute for Horticultural Development (IHD), Victoria, Australia

Mckinney HH (1923) Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *Journal of Agricultural Research* **26**, 195-219

Parlevliet JE (1979) Components of resistance that reduce the rate of epidemic development. *Annual Review of Phytopathology* **17**, 203-222

Paula Jr. TJ, Morandi MAB, Zambolim L, Silva MB (2005) Controle alternativo de doenças de plantas. In: Venezon M, Paula Júnior TJ, Pallini A (Eds) *Controle Alternativo de Pragas e Doenças*, EPAMIG/CTZM, Viçosa, pp 135-162

Rassoli I, Mirmostafa AS (2003) Bacterial susceptibility to and chemical composition of essential oils from *Thymus kotschyanus* and *Thymus persicus*. *Journal of Agricultural and Food Chemistry* **51**, 2200-2205

Santos GR, Zambolim L, Vale FXR, Maffia LA, Vieira JM (1994) Progresso e gradiente da ramulose do algodoeiro. *Fitopatologia Brasileira* **19**, 390-393

Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. *Phytopathology* **67**, 1051-1056

Somda I, Leth V, Séméré P (2007) Evaluation of lemongrass, eucalyptus and neem aqueous extraxts for controlling seed-borne fungi of sorghum grown in Burkina Faso. *World Journal of Agricultura Sciences* **3 (2)**, 218-223

Suassuna ND, Coutinho WM (2007) Manejo das principais doenças do algodoeiro no cerrado brasileiro. In: Freire EC (Ed) *Algodão no Cerrado do Brasil*, Associação Brasileira de Produtores de Algodão, Brasília, pp 479-522

Thanaboripat D, Monkontanawut N, Suvathi Y, Ruangrattanametee V (2004) Inhibition of aflatoxin production and growth of *Aspergillus flavus* by citronella oil. *KMITL Science Journal* **49 (1)**, 1-8

Valarini PJ, Frighetto RTS, Melo IS (1994) Potencial da erva medicinal *Cymbopogon citratus* no controle de fitopatógenos do feijoeiro. *Revista de Agricultura* **69**, 139-50

Yang P, Ma Y, Zheng S (2005) Adulticidal activity of five essential oils against *Culex pipiens Quinquefasciatus*. *Journal of Pesticide Science* **30 (2)**, 84-89