Insecticidal activity of *Tagetes* sp. on *Sitophilus zeamais* Mots

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***Abstract*—** *The indiscriminate use of agricultural inputs, such as fertilizers e and synthetic pesticides, can cause high levels of toxic residues in food, biological imbalance, environmental contamination, intoxication of humans and animals, and other direct and indirect effects. The use of plant extracts as alternative insecticides is a way to minimize the problems caused by synthetic insecticides. Insecticides are in the second position in the trading market of agrotoxics in Brazil. The species Tagetes erecta L. and Tagetes patula L. have antioxidant properties, larvicidal, fungicidal, antimicrobial, nematicide and insecticide. In order to verify the possibility of Tagetes sp. extracts be suitable alternative to the use of synthetic insecticides, the bioassays laboratorial were designed through of insect mortality test of Sitophilus zeamais. We evaluated the antioxidant activity by the test of DPPH, in addition to screen the chromatographic profile of the extracts. It is concluded that the extracts evaluated are efficient in insect mortality, checking still considerable amount of antioxidant compounds, as identified also flavonoids, terpenes and alkaloids in extracts. According to these results we conclude that T. erecta and T. patula has phytotoxic compounds that can promote and expand its use as a natural insecticide.*

***Keywords*— Alternative insecticides, natural defensive, organic agriculture, plant extracts**.

I. INTRODUCTION

The world population growth has led to increased food production demand (Menezes, 2005; Corrêa and Salgado, 2011), resulting in a green revolution, dating from the 1960s, which led to agriculture to be characterized as extensive monocultures and great use of synthetic fertilizers and agrotoxics, the latter being mainly composed of synthetic herbicides and insecticides (Menezes, 2005).

In relation to pesticides, several properties must be associated with the activity, such as effectiveness at low concentrations, no toxicity front of mammals and higher animals, easily obtainable, handling and application, economic feasibility and not be accumulated in human adipose tissue and also selective (Addor, 1994; Viegas Júnior, 2003). Within the insecticides classification are also included substances that repel and attract insects (Viegas Júnior, 2003).

Insecticides are in the second position in the trading market of agrotoxics in Brazil, with 25%, behind herbicides with 48% (Agrow, 2007; Ibama, 2009; Tavella et al., 2011). Insects are the major cause of losses in crops, especially grains and seeds, as they reduce their nutritional and commercial values, these attacks can occur before, during and after crop (Almeida et al., 2005). To date Brazil has its economy based on primary sector production, occupying a prominent position on the world supply of cereals, fruits and other products of plant origin (Viegas Júnior, 2003).

The maize weevil *Sitophilus zeamais* Mots., 1855 (Coleoptera: Curculionidae), is a cosmopolitan insect, of cross-infestation, means that the insect attack seeds in the field and also in warehouses (Almeida et al., 1999; Lorini et al., 2010; Antunes et al., 2011), have high reproductive potential, is considered an internal primary pest (Lorini et al., 2010; Antunes et al., 2011), causing serious economic losses (Restello et al., 2009, Antunes et al., 2011), because it has many hosts, such as wheat, corn, rice, barley and oats. Corn is one of the most important products of the agricultural sector in Brazil (Embrapa, 2010), is also considered the culture more attacked by *S. zeamais*, which in turn is reported as the most widespread and destructive species of cereals held in the world (Capps et al., 2010; Almeida et al., 2012).

Today we know that the frequent and indiscriminate use of chemicals, which are not always effective often cause the presence of high levels of toxic residues in food, of biological unbalance, environmental contamination, Intoxication humans and animals (Almeida et al. 1999; Lima et al., 2008; Marcomini et al, 2009; Queiroga et al, 2012), resurgence of pests and

strains of resistant insects (Dequech et al, 2008; Almeida et al, 2012), among other direct and indirect effects (Lima et al, 2008). It is also known that the use of plant extracts, as an alternative insecticide, is a way to provide a control without triggering the problems caused by chemical synthetic insecticides (Almeida et al., 1999).

An alternative to attenuate these problems is to use allelochemicals extracted from plant (Dequech et al., 2008). These factors justify the studies to identify management practices that reduce the use of synthetic products such as cultural practices based on allelopathy (Balbinot-Junior, 2004). The genus *Tagete*s, family Asteraceae , is used as an alternative for the control of pests and diseases (Salinas-Sánchez et al., 2012), and due to the chemical composition of their secondary metabolites, their biology activity have provided the development of new drugs and insecticides, among others (Verdi et al., 2005; Duque, 2006); is native to Mexico and Central America (Marotti et al., 2004; Peres, 2007; Santos, 2013), has therapeutic properties that have been recognized since the time of the Aztecs, being used to combat various diseases (Rondón et al., 2006; Jain et al, 2012; Tonuci et al, 2012). Among secondary metabolites found in the species *Tagetes* are: alilanisol, anetol, limonene, methyl eugenol, and β-karyophyllene that are have toxic to insects, mites, nematodes, bacteria, fungi, and viruses. Such compounds have been reported to be present in *Tagete*s essential oil, and they belong to certain groups of hydrocarbons, alcohols, ethers, aldehydes, ketones, esters, carotenoids (Rondón et al., 2006; Jain et al., 2012; Salinas-Sánchez et al., 2012; Santos, 2013), flavonoids, terpenes (Santos, 2013) and thiophenes (Duque, 2006; Dasgupta et al., 2012). The species *Tagetes erecta* L. e *Tagetes patula* L have antioxidant properties, larvicidal, fungicidal (Lopes et al, 2009; Martinez et al, 2009; Restello et al., 2009; Tonuci et al, 2012;) antimicrobial, nematicide, insecticide (Restello et al., 2009; Barboza et al., 2010).

Considering these perspectives, the aim of the study was evaluating the effects of hydroethanolic and ethanol extracts of *Tagetes erecta* L. and *Tagetes patula* L. (Asteraceae), on *Sitophilus zeamais*.

II. MATERIAL AND METHOD

2.1. Vegetal material and extract preparation

The vegetative parts of *T. patula* and *T. erecta* were collected from specimens grown in the field in Agência Paulista de Tecnologia dos Agronegócios (APTA) - Polo Regional Médio Paranapanema (22º37'07.92" S and 50º22'26.85' W, with altitude 701m). For preparation of extracts plant parts were washed, dried in an oven (40°C) and sprayed. The hydroethanolic extract was obtained by mechanical stirring in a solution of ethanol: water (70:30) at a ratio of 1:10 (w/v) for 24h at room temperature, and the process was repeated three times with the same plant material. Then, the extract was filtered and rotary evaporated (model MA120, Marconi, Brazil) at 60°C to remove the ethanol and was subsequently frozen and lyophilized to obtain the dry extract. Similarly, the ethanol extract was obtained by replacing the ethanol:water solution (70:30) for absolute ethanol (Impex, Brazil), being that the dried extract was obtained after concentration on a rotary evaporator followed by drying at room temperature.

2.2. Test insecticide of extracts of *Tagetes sp*.

2.2.1. Obtaining and Breeding of Insects

The species of insect *Sitophilus zeamais* was used. The insects were bred in wide-mouth plastic bottles with a capacity of one liter. The bottles were sealed with a fine mesh and inside grains of corn, such as food substrate to insects. The insects were separated randomly and kept without food for three hours before the preparation of bioassays, as suggested by Prates e Santos (2000).

2.2.2. Insecticide evaluation by application topical

We randomly selected 20 adults of *S. zeamais* and these put in *Petri* dishes containing 20 corn seeds were previously sterilized with sodium hypochlorite solution to 10%. After sterilization, the seeds were sprayed with the extracts (experimental groups) and with water (control group), the procedure was repeated every 24 hours for both.

The experiment was a completely randomized design (CRD) with four replications for each experimental and control groups. The treatments consisted of ethanolic and hydroethanolic extracts in the concentrations of 12.5, 25, 50, 100 and 200 $mg.mL^{-1}$

and control group using distilled water. The test was conducted in controlled conditions in BOD, temperature 25±2 °C, humidity of 70 ± 2% and photoperiod of 12 hours.

For the implementation of the solutions we used a hand sprayer with a capacity of 200 mL, ensuring application of 1.5 ± 0.5 mL per cm^2 of the *Petri* dishes.

The calibration of sprayer was performed by applying distilled water on filter paper discs were weighed before and after application. The mean of difference between the weighings was used as a standard value, according to the methodology established by members of the IOBC (Hassan, 1997). The monitoring of the experiment was carried out every 12 hours for 96 hours. During the experiment was evaluated the insect mortality.

2.2.3. Statistical analysis

The estimates of the lethal concentrations (LC_{50} e LC_{90}) were obtained through a Probit analysis (Finney, 1971).The software used for this purpose was Statgraphics Plus 5.0 (Statistical Graphics Corp., Fairfax, VA, U.S.A.).

2.3. Stable DPPH free radical scavenging activity

The stable 1.1-diphenyl-2-picrylhydrazyl (DPPH, Sigma, USA) radical scavenging activity was determined by Blois's method (1958). The extracts of each sample were dissolved in absolute ethanol at different concentrations (250, 500, 1000, 2000, and 4000 $\mu g.ml^{-1}$) and then mixed with 250µL solution of DPPH (500µM). The extracts reacted with the DPPH radical for a period of 30min at a low luminosity and were then submitted to the UV–vis spectrophotometer (Femto-600 Plus) at a 517nm wave length. The calculation of the antioxidant activity was performed according to the formula: antioxidant activity (%)=[(control-sample)/control]x100. The effective concentration and quantity of antioxidant required to decrease the initial concentration of DPPH by 50% (EC50) was estimated from an exponential curve is obtained by plotting on the abscissa the concentration of the sample ($mg.mL^{-1}$), or positive control and the ordinate, the percentage of antioxidant activity. Gallic acid (Vetec-QuímicaFina, Brazil) was used as the reference. Three repetitions were performed.

2.4. Chromatographic profile of the extracts

Thin layer chromatography (TLC) was performed for separation and identification of compounds from different extracts of *T. erecta* and *T. patula*. For TLC analysis was performed an optimization of methodology described by Wagner and Bladt (1996), extracts were diluted in the methanol, at a concentration of 100 $mg.mL^{-1}$. After this procedure, was applied 20ul of each sample in the TLC plates F250 (10cm x 10cm - MERCK).

The mobile phase for the detection of flavonoid was used eluent system chloroform-methanol-water (75:23:2) and standards were quercetin, rutin and gallic acid. The revelation of the plates was performed by spraying of solution polyethylene glycol (NP/PEG).

The different chromatographies were examined in ultraviolet (254 and 366nm) and after setting the chromatographic zones were calculated respective retention factors (Rf) using the following formula: Rf =Zcm/FRONT cm.

III. RESULTS

3.1. Insecticidal activity

The results for the evaluation of potential insecticide in the different concentrations of hydroethanolic and ethanolic extract of *T. erecta* and *T. patula* are shown in Table 1. The CL50 decreased progressively with increasing time of exposure to the various treatments being more marked between 12-24 hours. The smallest CL50 were observed in the time of 12 hours of treatment with hydroethanolic extract of *T. erecta* with a value of 17,07 $mg.mL^{-1}$ and for time of 24 and 36 hours on the hydroethanolic extract of *T. patula* with value of 11.43 and 10.88 $mg.mL^{-1}$ respectively.

After 60 hours from the beginning of the test, all treated with *T. erecta* extract reached a maximum value for mortality (80% of dead insects), because the treatments with *T. patula* reached its peak of mortality in 84 hours (Figure 1A e B).

TABLE 1
INSECTICIDAL ACTIVITY OF THE EXTRACT OF *TAGETES ERECTA* AND *TAGETES PATULA* AGAINST *SITOPHILUS ZEAMAIS* ADULTS.

Species	Extract	Duration (h)	LC_{50} (95% confidence interval) (mg/mL)	LC_{95} (95% confidence interval) (mg/mL)	Degrees of freedom	Chi-square	Slope±SE	Intercept±SE
T. erecta	Hydroethanolic	12	17.07 (12.64-20.53)	47.21 (41.01-57.67)	21	10	6.3±1.20	-16.02±2.6
		24	12.82 (10.58-14.50)	24.07 (21.50-28.60)	21	8	3.3±0.25	-11.17±3.9
		36	12.59 (10.75-14.02)	21.70 (19.31-26.32)	21	8	2.9±0.93	-10.04±2.1
	Ethanolic	12	18.69 (11.75-23.92)	68.65 (58.51-86.13)	21	10	5.1±1.12	-7.89±2.6
		24	12.14 (3.84-17.40)	55.21 (46.64-71.14)	21	8	2.4±1.01	-6.76±1.2
		36	11.23 (6.58-13.88)	28.78 (24.90-36.83)	21	8	2.8±1.23	-5.43±0.9
T. patula	Hydroethanolic	12	19.27 (15.13-22.72)	50.30 (43.87-60.89)	21	10	4.5±2.09	-11.09±2.3
		24	11.43 (6.75-14.12)	29.57 (25.52-37.95)	21	8	3.6±1.87	-8.16±3.6
		36	10.88 (6.39-13.43)	27.16 (23.61-34.39)	21	8	2.8±0.78	-7.09±2.5
	Ethanolic	12	27.92 (23.15-32.47)	72.14 (62.65-87.41)	21	13	7.6±0.98	-19.14±3.4
		24	17.56 (11.80-21.87)	56.80 (48.70-70.90)	21	10	3.9±1.16	-16.09±2.1
		36	12.59 (10.75-14.02)	21.70 (19.31-26.32)	21	8	3.5±1.12	-11.36±3.5

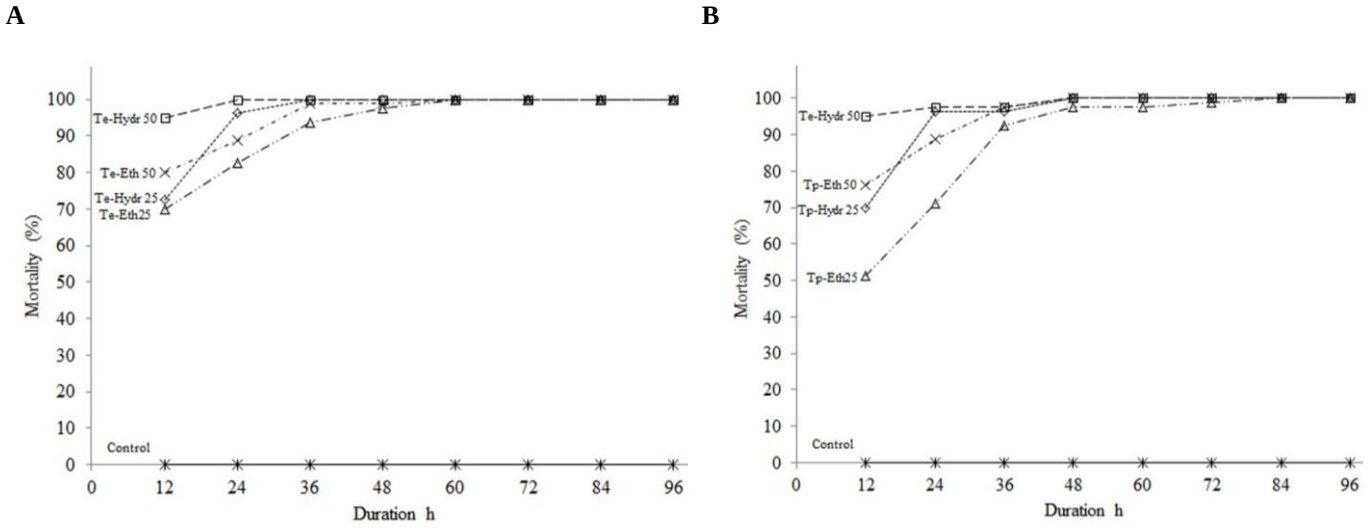

FIGURE 1 - PERCENTAGE OF DEAD INSECTS BY THE TIME IN HOURS AFTER SPRAYING HYDROETHANOLIC AND ETHANOLIC EXTRACTS OF *T. ERECTA* (A) AND *T. PATULA* (B), IN TWO DOSES FOR EACH TREATMENT.

3.2. Antioxidant activity

In the method of DPPH radical scavenging free stable activity the highest activities observed for *T. erecta* and *T. patula* extracts were for the concentration of 4000 $\mu g.mL^{-1}$ (Table 2). The hydroethanolic extract of *T. erecta* showed 81.43% and the ethanolic was 74.89%. For *T. patula*, the values observed for the hydroethanolic and ethanolic extracts were 79.76% and 72.06% respectively. In relation to the EC50 values, the ethanolic extracts of *T. erecta* and *T. patula* had higher antioxidant potential, with values of 1793.73 and 1947.21 $\mu g.mL^{-1}$ respectively (Table 2).

TABLE 2.
ANTIOXIDANT ACTIVITY BY DPPH METHOD AND EC_{50} OF AQUEOUS EXTRACTS, HYDROETHANOLIC AND ETHANOLIC *T. ERECTA* AND *T. PATULA*.

Extract (µg/mL)	(%) DPPH			
	T. erecta		*T. patula*	
	Hydroethanolic	Ethanolic	Hydroethanolic	Ethanolic
250	05.63±0.70	00.76±0.26	09.31±3.60	10.80±3.44
500	08.07±0.53	10.05±1.99	15.79±1.07	15.38±3.64
1000	25.57±1.21	22.68±1.47	28.21±1.64	24.97±4.08
2000	48.10±0.70	57.23±0.70	41.16±3.67	52.23±4.38
4000	81.43±1.15	74.89±1.58	79.76±1.07	72.06±0.40
EC_{50}	2245.68	1793.73	2373.96	1947.21

3.3. Profile chromatographic

In the chromatogram in the TLC revealed with reagent NP/PEG subjected to ultraviolet light, which were applied and eluted samples hydroethanolic and ethanolic extract of *T. patula* (TpH and TpE respectively) and *T. erecta* (TeH and TeE respectively), along with the standards, quercetin, rutin and gallic acid. The presence of polifenolis was performed by chromatographic elution and calculations of values Rfs to different samples (Fig 2).

The Rfs of quercetin and gallic acid standards were 0,66 and 0,24 respectively, and the rutin was 0,11. The hydroethanolic extract of *T. patula* presented the Rfs 0.22 and 0.65 cm, values similar to those found in the quercetin and rutin standards, already in the ethanolic extract of the same plant was observed the Rf 0.23 cm, similar to the gallic acid standard.

The hydroethanolic and ethanolic extracts of *T. erecta* showed the values of Rfs 0,22 and 023 cm respectively, similar to the Rfs of gallic acid standard. Despite the similarity of Rf s, we can not affirm the presence of standard in the samples because it was not observed the same colorations in Rfs analyzed in comparison to standards, with the exception of rutin, with yellow coloration present in all extracts (Fig 2).

In the different samples analyzed occur also Rfs different of standards used for chromatographic comparison, thus suggesting the presence of different classes of polyphenols in the plant extracts.

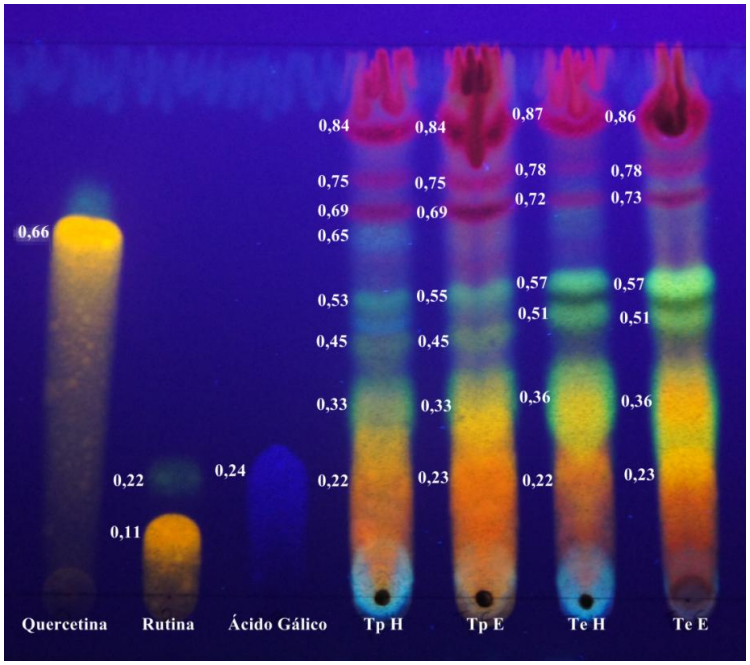

FIGURE 2. CHROMATOGRAM OF DIFFERENT EXTRACTS OF *T. ERECTA* (HYDROETHANOLIC=TE H, ETANOLIC=TE E), *T. PATULA* (HYDROETHANOLIC=TE H, ETANOLIC=TE E), QUERCETIN, RUTIN AND GALLIC ACID STANDARDS, AS ELUENT SYSTEM CHLOROFORM-METHANOL-WATER (75:23:2) AND DEVELOPER NP/PEG, FOR IDENTIFICATION OF FLAVONOID COMPOUNDS. CHROMATOGRAPHIC IDENTIFICATION OF AREAS WITH THEIR RF VALUES (CM).

IV. DISCUSSION

In the evaluation of insecticide bioassay performed in this study, was observed the insecticide effect of *T. erecta* and *T. patula* extracts on adult *Sitophilus zeamais*, under laboratory conditions. The different concentrations of the extracts evaluated, except the concentration of 12.5 $mg.mL^{-1}$, had a mortality rate of 100% at the end of 36 hours, while the control group showed no mortality until the end of the study.

According to Prates and Santos (2000), a possible mechanism of action for plant extracts is connected to "knockdown effect", this shock effect is characterized by insect inability to walk and progressing to death. Thus, similar results were observed by Nascimento et al., (2008), where 96-100% of adult *Sitophilus zeamais* died in *T. patula* extract when applied in vapor form; Restello et al (2009) showed 100% mortality of the insects with the use of essential oil of *T. patula*. In addition to the "knockdown effect", Prates and Santos (2000) reported that the most effective insecticides are action by contact and/or ingestion and fumigant because they are more effective on stored grain pests.

In particular for specie *T. erecta*, in studies conducted by Marcomini et al., (2009), there were no significant results, regarding the use of essential oils from flowers in mortality *Alphitobius diaperinus*. Salinas-Sánchez et al., (2012) tested different extracts of *T. erecta* in mortality of *Spodoptera frugiperda* in different stages, obtaining better results for the ethanolic extract of the leaves. Therefore, it is found in this study the efficacy insecticide of *T. erecta* on the kind of target insect. Keita et al., (2000), in studies with species of the genus Tagetes have insecticidal activity against pests of stored products.

Recent studies by Ben El Hadj Ali et al. (2015), Ulukanli et al. (2014) and Khiyari et al. (2014) have shown that plant species that exhibit insecticidal compounds may be correlated with the presence of different phenolic compounds in its composition. The activity of these compounds is proven with the determination of antioxidant potential.

Evaluation of antioxidant activity of different extracts of *T. erecta* and *T. patula* was measured by verifying an increase, depending on the concentration, for the DPPH test. The scavenging activity of DPPH radical may be related to the content of certain phenolic compounds, including flavonoids. Similar results were demonstrated in studies carried out by Cetkovic et al. (2004), Siriamornpun et al. (2012) and Gong et al. (2012) in different species of genus *Tagetes*. In studies conducted by Toscan (2010), Sá (2011) and Jain et al., (2012) using plant extracts is observed that plants with insecticidal activity also showed antioxidant potential, therefore these studies are in accordance with this work.

For chromatographic tests, according to Wagner and Bladt (1996), the fluorescence seen in wavelength of 254nm indicates the presence of the group of flavonoid compounds and in the wavelength of 365nm, depending on the structural type. Flavonoids reflects the dark yellow color, green or blue fluorescence, which is the case of gallic acid standard, used in chromatographic plate, whose presence was not observed in the extracts analyzed, the blue coloration observed in the treatment indicates the presence of other phenolic acids. The analysis of Rfs and colorations, orange yellow and fluorescent orange, observed in treatments only indicate the presence of rutin standard, greenish yellow coloration may indicated the presence specific flavonoids, such as flavones. According to Schaller (2008) flavonoids are among the classes of plant secondary metabolites with known insecticidal activity, and these compounds are known to confer protection to the plant against herbivores.

V. CONCLUSION

Considering the results, this study showed that *T. erecta* and *T. patula* extracts has insecticidal effect on *Sitophilus zeamais* and may come to replace synthetic products and is used as an alternative method, improving efficiency in pest control, reducing economic losses and enabling greater sustainability to the agricultural system.

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REFERENCES

[1] Addor, R.W., 1994. In Agrochemical from Natural Products; Godfrey, C.R.A., ed.; Marcel Dekker Inc.: New York.
[2] AGROW, 2007. Complete guide to generic pesticides. Disponível em: <http://www.agrow.com/multimedia/archive/00053/DS258_58994a_53150a.pdf> Acesso em: Março/2014.

[3] Almeida, F.A.C., Goldfarb, A.C., Gouveia, J.P.G., 1999. Avaliação de extratos vegetais e métodos de aplicação no controle de *Sitophilus* sp. Revista Brasileira de Produtos Agroindustriais, Campina Grande, v.1, n.1, p.13-20.
[4] Almeida, F.A.C., Pessoa, E.B., Gomes, J.P., Silva, A.S., 2005. Emprego de extratos vegetais no controle das fases imatura e adulta do *Sitophilus zeamais.* Revista Agropecuária Técnica, v.26, n.1.
[5] Almeida, F.A.C., Silva Júnior, P.J., Silva, J.F., Lino, T.F.L., Silva, R.G., 2012. Infestação e germinação em sementes de milho tratadas com extratos de *Piper nigrum* e *Annona squamosa*. Revista Brasileira de Produtos Agroindustriais, Campina Grande, v.14, n. Especial, p.457-471.
[6] Antunes, L.E.G., Viebrantz, P.C., Gottardi, R., Dionello, R.G., 2011.Características físico-químicas de grãos de milho atacados por *Sitophilus zeamais* durante o armazenamento. Revista Brasileira de Engenharia Agrícola e Ambiental, v.15, n.6, p.615-620.
[7] Balbinot-Junior, A.A., 2004. Manejo das plantas daninhas pela alelopatia. Agropecuária Catarinense, Florianópolis, v.17, n.1, p.61-64.
[8] Barboza, V.C., Vieira, M.C., HerediaZárate, N.A., Padovezzi, V.H., Santos, M.J.G., 2010. Cama-de-frango em mono e policultivo de fáfia com cravo-de-defunto e manjericão. Revista Horticultura Brasileira, n.28, p.348-354.
[9] Ben El Hadj Ali, I., Chaouachi, M., Bahri, R., Chaieb, I., Boussaïd, M., Harzallah-Skhiri, F., 2015. Chemical composition and antioxidant, antibacterial, allelopathic andinsecticidal activities of essential oil of *Thymus algeriensis* Boiss. Et Reut. Industrial Crops and Products, v.77, p. 631–639.
[10] Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature, v.181, p.1199-1200.
[11] Capps, A.L.A.P., Novo, J.P.S., Novo, M.C.S.S., 2010. Repelência e toxicidade de *Cyperusiria* L., em início de florescimento, ao gorgulho *Sitophilus oryzae*. Revista Brasileira de Engenharia Agrícola e Ambiental, v.14, n.2, p.203-209.
[12] Cetkovic, G.S., Djilas, S.M., Canadanovic-Brunet, J.M., Tumbas, V.T., 2004. Antioxidant properties of marigold extracts. Food Research International v.37, p.643-650.
[13] Corrêa, J.C.R., Salgado, H.R.N., 2011. Atividade inseticida das plantas e aplicações: revisão. Revista Brasileira de Plantas Medicinais, Botucatu, v.13, n.4, p.500-506.
[14] Dasgupta, N., Ranjan, S., Saha, P., Jain, R., Malhotra, S., Saleh, M.A.A.M., 2012. Antibacterial activity of leaf extract of mexican marigold (*Tagetes erecta*) against diferente gram positive and gram negative bacterial strains. Journal of Pharmacy Research, n.5(8), p.4201-4203.
[15] Dequech, S.T.B., Sausen, C.D., Lima, C.G.; Egewarth, R., 2008. Efeito de extratos de plantas com atividade inseticida no controle de *Microtheca ochroloma* Stal (Col.: Chrysomelidae), em laboratório. Revista Biotemas, n.21(1), p.41-46.
[16] Duque, M.M.M.M., 2006. *Tagetes erecta* L.. Uma planta de múltiplos propósitos: antibacteriana, larvicida e antiviral. Dissertação (Mestrado Acadêmico em Ciências Fisiológicas), Universidade Estadual do Ceará, Centro de Ciências da Saúde, Fortaleza.
[17] Embrapa Empresa Brasileira de Pesquisa Agropecuária - Agência de informação Embrapa milho, 2010.
[18] Estrela, J.L.V., Fazolin, M., Catani, V., Alécio, M.R., Lima, M.S., 2006. Toxicidade de óleos essenciais de *Piper aduncum* e *Piper hispidinervum* em *Sitophilus zeamais*. Pesquisa Agropecuária Brasileira, Brasília, v.41, n.2, p.217-222.
[19] Finney, D.J., 1971. Probit Analysis. Cambridge University Press, Cambridge, p. 31.
[20] Gong, Y., Liu, X., He, W.H., Xu, H.G., Yuan, F., Gao, Y.X., 2012. Investigation into the antioxidant activity and chemical composition of alcoholic extracts from defatted marigold (*Tagetes erecta* L.) residue. Fitoterapia, v.83, p.481-489.
[21] Hassan, S.A., 1997. Métodos padronizados para testes de seletividade, com ênfase em *Trichogramma*. In: Parra, J.R.P.; Zucchi, R.A. (Eds.) *Trichogramma* e o controle biológico aplicado. FEALQ, Piracicaba, p.207-234.
[22] IBAMA. Produtos agrotóxicos e afins comercializados em 2009 no Brasil. 2009. Disponível em:<http://www.ibama.gov.br/qualidade-ambiental/wpcontent/files/ Produtos_Agrotoxicos_Comercializados_Brasil_2009.pdf>. Acesso em: Março/2014.
[23] Jain, R., Katare, N., Kumar, V., Samanta, A.K., Goswami, S., Shrotri, C.K., 2012. In vitro antibacterial potential of diferente extracts of *Tagetes erecta* and *Tagetes patula*. Journal of Natural Sciences Research, v.2, n.5.
[24] Keita, S.M., Vicent, C., Schmit, J., Ramaswamy, S., Belanger, A., 2000. Effect of various essential oils on *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae). Journal of Stored Products Research, v.36, p.355-364.
[25] Khiyari, M.E.A., Kasrati, A., Jamali, C.A., Zeroual, S., Markouk, M., Bekkouche, K., Wohlmuth, H., Leach, D., Abbad, A., 2014. Chemical composition, antioxidant and insecticidal properties of essential oils from wild and cultivated *Salvia aucheri* subsp. *blancoana* (Webb. & Helder)), an endemic, threatened medicinal plant in Morocco. Industrial Crops and Products, v.57, p.106–109.
[26] Lima, J.F.M., Grützmacher, A.D., Cunha, U.S., Porto, M.P., Martins, J.F.S., Dalmazo, G.O., 2008. Ação de inseticidas naturais no controle de *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae) em milho cultivado em agroecossistema de várzea. Revista Ciência Rural, v.38, n.3.
[27] Lopes, R.K., Ritter, M.R., Rates, S.M.K., 2009. Biological activities and toxicity of ornamental plants of Rio Grande do Sul: a review. Revista Brasileira de Biociências, n.7, p.305-315.
[28] Lorini, I., Krzyzanowski, F.C., França-Neto, J.B., Henning, A.A., 2010. Principais pragas e métodos de controle em sementes durante o armazenamento – Série Sementes. Circular Técnica – Embrapa Soja, n.73.
[29] Marcomini, A.M., Alves, L.F.A., Bonini, A.K., Mertz, N.R., Santos, J.C., 2009. Atividade inseticida de extratos vegetais e do óleo de nim sobre adultos de *Alphitobius diaperinus* Panzer (Coleoptera, tenebrionidae). Arquivos do Instituto Biológico, São Paulo, v.76, n.3, p.409-416.
[30] Marotti, M., Piccaglia, R., Biavati, B., Marotti, I., 2004. Characterization and yield evaluation of essential oils from different *Tagetes* species. Journal Essential Oil Research, v.16, p.440-444.
[31] Martinez, R., Diaz, B., Vasquez, L., Compagnone, R.S., Tillett, S., Canelon, D.J., Torrico, F., Suarez, A.I., 2009. Chemical composition of essential oils and toxicological evaluation of *Tagetes erecta* and *Tagetes patula* from Venezuela. Journal Essential Oil Research.n.12, p. 476-481.
[32] Menezes, E.L.A, 2005. Inseticidas botânicos: seus princípios ativos, modo de ação e uso agrícola. Seropédica, Rio de Janeiro: Embrapa Agrobiologia, p.58.
[33] Nascimento, F.J., Diniz Filho, E.T., Mesquita, L.X., Oliveira, A.M., Pereira, T.F.C, 2008. Extractos Vegetales en el Control de Plagas. Revista Verde, n.3, p.01-05.

[34] Peres, F.S.C, 2007. Cravo-de-Defunto (*Tagetes patula* L.) como planta atrativa para tripes (Thysanoptera) e himenópteros parasitóide (Hymenoptera) em cultivo protegido. Dissertação (Mestrado) - Universidade Estadual Paulista, Faculdade de Ciências Agrárias Veterinárias - Unesp, Campus de Jaboticabal.
[35] Prates, H.T., Santos, J.P. Óleos essenciais no controle de pragas de grãos armazenados, p. 443-461. *In*: Lorini, I.; Miike, L.H.; Scussel, V.M, 2000. Armazenagem de Grãos. Campinas: IBG, p.1000.
[36] Queiroga, M.F.C., Gomes, J.P., Almeida, F.A.C., Pessoa, E.B., Alves, N.M.C, 2012. Aplicação de óleo no controle de *Zabrotes subfasciatus* e na germinação de *Phaseolus vulgaris*. Revista Brasileira de Engenharia Agrícola e Ambiental, v.16, n.7, p.777-783.
[37] Restello, R.M., Menegatt, C., Mossi, A.J, 2009. Efeito do óleo essencial de *Tagetes patula* L. (Asteraceae) sobre *Sitophilus zeamais* Motschulsky (Coleoptera, Curculionidae). Revista Brasileira de Entomologia, v.53, n.2, p.304-307.
[38] Rondón, M., Velasco, J., Hernández, J., Pecheneda, M., Rojas, J., Morales, A., Carmon1 R., Díaz, T. 2006. Chemicalcompositionandantibacterialactivityoftheessentialoilof*Tagetes patula* L. (Asteraceae) collectedfromthe Venezuela Andes. Revista Latinoamericana de Química, n.34, p.1-3.
[39] Sá, C.G, 2011. Extração e testes de atividades farmacológicas do óleo essencial de *Citrus sinensis* (L.) Osbeck direcionados para a doença de Alzheimer. Dissertação (Mestrado - Programa de Pós-Graduação em Ciências Farmacêuticas).
[40] Salinas-Sánchez, D.O., Aldana-Llanos, L., Valdés-Estrada, M.E., Gutiérrez-Ochoa, M., Valladares-Cisneros, G., Rodríguez-Flores, E, 2012. Insecticidal activity of *Tagetes erecta* extracts on *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Florida Entomologist, v.95, n.2, p.428-432.
[41] Santos, J.M, 2013. Caracterização Química e efeito acaricida do óleo essencial de *Tagetes minuta* Linnaeus (Asteraceae) em diferentes espécies de carrapatos. Dissertação (Mestrado, Pós-Graduação Em Ciência Animal), Universidade Federal De Mato Grosso Do Sul, Campo Grande/MS.
[42] Schaller, A, 2008. Induced plant resistance to herbivory. Hardcover: Springer, p.464.
[43] Siriamornpun, S., Kaisoona, O., Meeso, N, 2012. Changes in colour, antioxidant activities and carotenoids (lycopene, b-carotene, lutein) of marigold flower (Tagetes erecta L.) resulting from different drying processes. Journal of Functional Foods, v.4, p.757-766.
[44] Tavella, L.B., Silva, I.N., Fontes, L. O., Dias, J.R.M., Silva, M.I.L, 2011. O uso de agrotóxicos na agricultura e suas consequências toxicológicas e ambientais. ACSA - Agropecuária Científica no Semiárido, v.7, n.2, p.06-12.
[45] Tonuci, L.R.S., Melo, N.I., Dias, H.J., Wakabayashi, K.A.L., Aguiar, G.P., Aguiar, D.P., Mantovani, A.L.L., Ramos, R.C., Groppo, M., Rodrigues,V., Veneziani, R.C.S., Cunha, W.R., Silva Filho, A.A., Magalhães, L.G., Crotti, A.E.M, 2012. In vitro schistosomicidal effects of the essential oil of *Tagetes erecta*. Brazilian Journal of Pharmacognosy, v.22, n.1, p.88-93.
[46] Toscan, C.M., 2010. Atividade antimicrobiana e antioxidante de terpenoides. Dissertação (Mestrado Programa de Pós-Graduação em Biotecnologia da Universidade de Caxias do Sul).
[47] Ulukanli, Z., Karabörklü, S., Bozok, F., Ates, B.; Erdogan, S., Cenet, M., Karaaslan, M.G., 2014. Chemical composition, antimicrobial, insecticidal, phytotoxic and antioxidant activities of Mediterranean *Pinus brutia* and *Pinus pinea* resin essential oils. Chinese Journal of Natural Medicines,v.12(12), p.901-910.
[48] Vendramim, J.D., 1997. Uso de plantas inseticidas no controle de pragas. In: ciclo de palestras sobre agricultura orgânica. São Paulo. Palestras. Campinas: Fundação Cargill, p.64-69.
[49] Verdi, L.G., Brighente, I.M.C., Pizzolatti, M.G, 2005. Gênero Baccharis (Asteraceae): Aspectos Químicos, Econômicos e Biológicos. Química Nova, v.28, n.1, p.85-94.
[50] Viegas Júnior, C., 2003. Terpenos com atividade inseticida: uma alternativa para o controle químico de insetos. Revista Química Nova, v.26, n.3, p.390-400.
[51] Wagner, H., Bladt, S., 1996. Plant drug analysis: a thin layer chromatography atlas. secund ed. Springer Verlag, Berlim, p.384.