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Insecticidal effect of from three *Hypericum* species extracts against *Rhyzopertha dominica*, *Sitophilus oryzae* and *Tribolium confusum*

Efeito inseticida dos extratos de três espécies de *Hypericum* contra ***Rhyzopertha dominica***, ***Sitophilus oryzae*** e ***Tribolium confusum***

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ABSTRACT

The search for new plant natural products with insecticidal properties to control insect pests in agriculture has gained relevance in the past decades. The aim of the study was to investigate the insecticidal activity of extracts derived from flower, leaf, and stem of three *Hypericum* species (*Hypericum heterophyllum*, *Hypericum perforatum*, *Hypericum scabrum*) against the adults of three important stored grain insect pests namely; *Sitophilus oryzae* (Curculionidae), *Rhyzopertha dominica* (Bostrichidae) and *Tribolium confusum* (Tenebrionidae). The insects were incubated with the food under 10% concentration of *Hypericum* extracts and the mortality was recorded after 24, 48 and 72 h of exposure. The extracts of the *Hypericum* species and exposure time were found to have statistically significant effective against the three insect pests. After 72 h exposure, the mortality ranged from 4.3 to 94.1 % for all insects. Among tested insects, *R. dominica* was more susceptible than *T. confusum* and *S. oryzae*. Although desirable insecticidal effect against the insects were recorded from all the three *Hypericum* species, the leaf extract of *H. perforatum* was more effective on *R. dominica*, while the flower and stem of *H. scabrum* displayed high toxic effect on *T. confusum* and *S. oryzae*, respectively. The leaf extracts, of *H. perforatum*, in particular, may be used as source of new potential botanical insecticides against *R. dominica* in stored grains.

Index terms: *H. heterophyllum*; *H. scabrum*; *H. perforatum*; stored grain; insects.

RESUMO

A busca por novos produtos vegetais com propriedades naturais inseticidas para o controle de insetos nocivos à agricultura têm ganho relevância nas últimas décadas. O objetivo do estudo foi investigar a atividade inseticida extraída dos extratos derivados da flor, folha e caule de três espécies de *Hypericum* (*Hypericum heterophyllum*, *Hypericum perforatum* e *Hypericum scabrum*) contra os adultos de três importantes insetos nocivos aos grãos armazenados: *Sitophilus oryzae* (Curculionidae), *Rhyzopertha dominica* (Bostrichidae) e *Tribolium confusum* (Tenebrionidae). Os insetos foram incubados com o alimento sob a concentração de 10% dos extratos de *Hypericum* e a mortalidade foi registrada após 24, 48 e 72 horas de exposição. Os extratos das espécies de *Hypericum* e o tempo de exposição foi considerado estatisticamente significativo e eficaz contra os três insetos nocivos. Após 72 horas de exposição, a mortalidade variou de 4,3 a 94,1% para todos os insetos. Entre os insetos testados, *R. dominica* foi mais suscetível do que *T. confusum* e o *S. oryzae*. Embora, o efeito inseticida desejável contra os insetos tenha sido registrado em todas as três espécies de *Hypericum*, o extrato da folha de *H. perforatum* foi o mais eficaz em *R. dominica*, enquanto que a flor e o caule de *H. scabrum* exibiram alto efeito tóxico sobre *T. confusum* e *S. oryzae*, respectivamente. Os extratos da folha de *H. Perforatum*, em particular, pode ser usado como fonte de novos inseticidas botânicos com potenciais contra *R. dominica* em grãos armazenados.

Termos para indexação: *H. heterophyllum*; *H. scabrum*; *H. perforatum*; grãos armazenados; insetos.

INTRODUCTION

The *Hypericum* genus has about 500 species divided into 36 taxonomic sections using morphological characters (Robson, 2003; Zhou et al., 2020) and adapted to different climatic and ecological conditions (Sarrou et al., 2018). The genus including a large number of medicinal and aromatic species contain a wide variety of the important chemical compounds such as napthodianthrones (hypericin, pseudohypericin, protohypericin), phenolic acid (chlorogenic acid), flavonoids (hyperoside, rutin, quercetin, campherol, luteolin, hyperin), phloroglucinols (hyperforin, furohyperforin), essential oils and xanthones (Napoli et al., 2018). *Hypericum* plant extracts exhibit many pharmacological properties, such as antioxidant, antidepressant, antitumor, antibacterial, antimicrobial, and anti-inflammatory effects. Some *Hypericum* species have active usage in traditional and modern medicine due to very important biological activities including antioxidant, antidepressant, antitumor, antibacterial, antimicrobial, and anti-inflammatory effects (Zorzetto et al., 2015). Herbal samples of *H. perforatum* (St. John's Wort) are widely utilized for the treatment of mild to moderate depression in Europe and the US, which also indicates that the drug has advantages compared to synthetic antidepressants by several studies (Kasper et al., 2010; NG et al., 2017; Marrelli; Stattı; Confortı, 2020).

Sitophilus oryzae (Curculionidae), *Rhyzopertha dominica* (Bostrichidae) and *Tribolium confusum* (Tenebrionidae) are the major insects that cause significant damage to many products during the storage. The larvae and adults of *S. oryzae* feed on rice, wheat, barley, corn, sorghum and other grain products (Mason; McDonoug, 2012; Mishra; Pandey, 2014) Similarly, *R. dominica* feed on all kinds of cereal grains (wheat, corn, rice, sorghum), products made from grains, flour-made ingredients, walnuts, hazelnuts, dried figs and legume products (Dissanayaka et al., 2020). *Tribolium confusum* feeds on spills of grains, flour and flour products, bran, semolina, spices, dried fruits, vegetables and legumes (Ajayi; Oladıpupo; Ajısafe, 2019). These insects cause both qualitative and quantitative losses to cereal grains. Insect damage in stored products may cause losses of 5–10% in developed countries and up to 20–40% in developing countries (Hernandez Nopsa, 2015). In addition, it creates serious problems for the food industry due to food contamination (Wu et al., 2019).

Synthetic chemical pesticides and insecticides have been actively used to effectively control of stored grain insects for many years (Kostyukovsky; Shaaya, 2013). However, scientists have begun to search for new and natural products to combat against stored product insects due to some adverse effect such as toxicity to mammals, development of resistant strains of insect populations, change of ecological balance and high costs (Rouhani et al., 2019; Lampiri et al., 2020). Low-cost organic sources that are less harmful to the environment and human health are very valuable. Many researches have investigated insecticidal affects of different plant species (Guru-Pirasanna-Pandi et al., 2018; Lampiri et al., 2020). Since plants are natural products, they are safer agricultural products, and cause less pollution (Sodaeizadeh; Rafıeıolhossaını; Van Damme, 2010).

Many studies have shown that *H. perforatum* is effective as a larvicidal, insecticide, acaricides, antimicrobial, growth-inhibitory activity, as well as antifeeding and repellent properties (Da Silva et al., 2013; Binias; Gospodarek; Rusın, 2016; Erdoğan; Yıldırım, 2016; Lazzara; Carrubba; Napolı, 2019; Keser et al., 2020). Also, many researchers reported the toxic effects of many *Hypericum* extracts against several insects or their larvaes in previous studies. For example, Da Silva et al. (2013) recorded that hexane extract derived from flowering aerial parts of *H. carinatum* was highly toxic on larvaes of Aedes aegypti. Erdoğan and Yıldırım (2016) found that ethanolic extract of *H. calycinum* (12%) caused mortality against nymph and adult of *Myzus persicae* Sulzer under both leaf dipping and spraying methods. Puthur et al. (2019) reported that of 2000 mg/L concentration of *H. japonicum* methanol extract caused 85% mortality on *S. oryzae*. *Hypericum* extracts have been shown to exhibit potent toxic effect on insects, particularly against coleopteran insects (Tozlu et al., 2011; Dastagir; Ahmed; Shereen, 2016; Puthur et al., 2019). It has therefore been hypothesized that the extracts of these *Hypericum* species could potentially exhibit insecticidal activity against adults of the stored grain insects such as *S. oryzae, R. dominica* and *T. confusum*. But, there have been unsufficiently researched the insectidisal effect of *H. perforatum*, *H. heterophyllum*, *H. scabrum* species on these insects. Type and quantity of plant phytochemicals can vary according to different parts of the plant, which causes differences in effectiveness (Sarrou et al., 2018). The aim of this study was to evaluate the effect of flower, leaf and stem extracts of three *Hypericum* species against on important stored product insects such as *R. dominica, S. oryzae* and *T. confusum*.

MATERIAL AND METODS

Plant material

The plant species were identified by Prof. Dr. OsmanTugay (Department of Pharmaceutical Botany, Faculty of Pharmacy, Selçuk University). Voucher specimens are kept in KNYA Herbarium of the Selçuk University, Faculty of Science, Konya, Turkey (Herbarium numbers: 28281, 28282, and 28283, respectively). *Hypericum heterophyllum* Vent., *H. scabrum* L., and *H. perforatum* L. were collected at 100% flowering period from their natural habitats in Turkey (Table 1). Collection of plants was done between 11:00 and 13:00 hours. The flowers, leafsves and stems of the plants were separated, and dried under shade at 20 ± 2 ºC.

Table 1: Habitat and collection status of the *Hypericum* species.

Species	Location	Collection time	Latitude (N)	Longitude (E)	Altitude (m)
H. heterophyllum[1]	Yozgat, Bozok University Campus	04.07.2017	39°46'42	34°47'51	1332
H. perforatum	Çorum, Village	01.07.2017	40°41'16	34°7'51	1372
H. scabrum	Yozgat, Gelin Kayası	13.06.2017	39°50'20	34°45'44	1401

[1]It is endemic to Turkey.

Preparation of samples for analysis

The flower, leaf and stem of the *Hypericum* species were used for the extraction. The plant materials were dried under shade and mechanically ground with a blender. About 4 g of each grounded plant materials were extracted individually in 40 mL of 100% acetone at 40 °C for 24 h. The resulting solutions were filtered through whatman paper and the solvent was removed on a rotary evaporator at temperature bellow 40 °C. The extracts were dissolved with acetone. Finally, the concentration of the extracts was adjusted to 10%, and all samples were stored at 4 ºC until used.

Test insects

Stock cultures of insects were obtained from the Department of Plant Protection, Yozgat Bozok University. Wheat grains were used for feeding of *S. oryzae* and *R. dominica* adults, and crushed wheat grains were used for feeding of *T. confusum* adults. In order to feed the insect species used in the study, 1-liter jars were filled with 1/3 food. Adult individuals were taken into these jars and left to feed. These stock cultures were incubated at 27±2 ºC in dark conditions. Within 45 days, a new generation of adults emerged, and randomly selected adult individuals were used in the study (Abay et al., 2012).

Contact toxicity

The 10% concentration of *Hypericum* extracts were used for contact effect according to method used by Gökçe et al. (2010). About 1 µl of the extracts was applied topically to each insect by micro-aplicator (Figure 1). For control, insects were topically treated with 1 µl of acetone solvent. The experiment was set up in complete randomized block design with six replications of ten insects pre replicate in a petri dish containing food. Insect mortality was recorded after 24, 48 and 72 h of exposure. The mortality rates were calculated as %.

Data analysis

No mortality was observed in the control applications of all species. The same vials were examined for mortality at the different exposure intervals (24, 48, and 72 hours), so mortality data were analyzed by using one-way analysis of variance (ANOVA) with *Hypericum* specie, plant part and exposure time as the main effects. Percent mortality data were expressed as mean values and standard error (±SE). It was used Levene's test to check homogenity of variances before ANOVA tests. Also, data given in percentages were subjected to arcsine ($\sqrt{X}$) transformation before statistical analysis. Differences between the means were compared by Duncan's multiple range tests using SPSS 20.0 Statistical software at 0.01 level.

RESULTS AND DISCUSSION

Mortality of *R. dominica* treated with the *Hypericum* extracts

The results from the individual effect of each factor (*Hypericum* specie, plant part and exposure time) had a statistically significant effect against *R. dominica* adults ($P < 0.01$) (Table 2). In terms of the interactions of these main factors, all interactions within exposure time had no statistically significance ($P > 0.80$), whereas the interaction between *Hypericum* specie and plant part shown a highly significant effect ($F=38.96$, $P < 0.01$).

According to the results obtained in Table 3, mortality of *R. dominica* was recorded the highest in leaf of *H. perforatum* (88.0%), and found to increase with the exposure of application in all extracts. After 72 h of exposure, the leaf of *H. perforatum* was exhibited the highest mortality with 94.1% against this pest.

In screening bioassays of different *Hypericum* species, *H. perforatum* (42.3%) were found to be more effective against *R. dominica* than *H. scabrum* (31.4%) and *H. heterophyllum* (31.0%). When the difference between plant parts is examined, the leaf exhibited the highest toxicity on *R. dominica* (48.8%) (Figure 2).

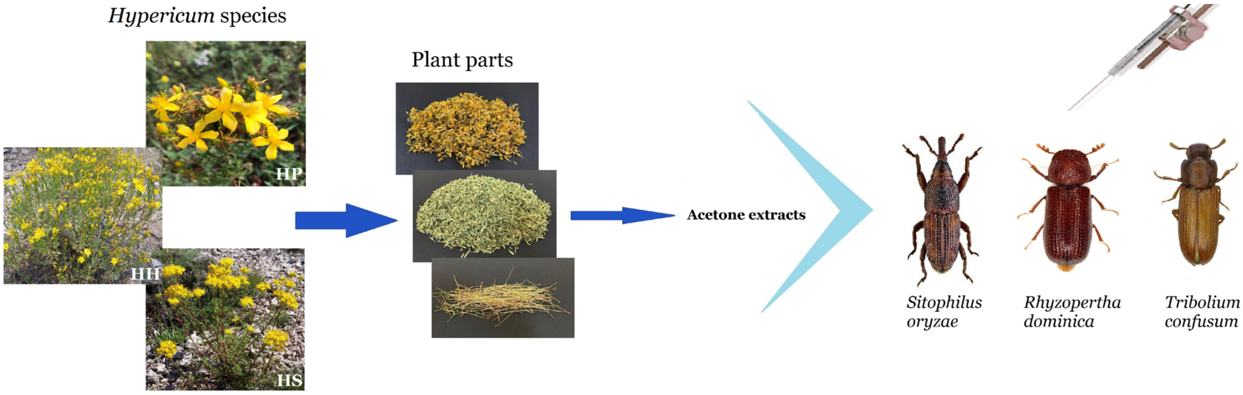

Figure 1: Application of *Hypericum* extracts on stored product insects (HP:*H. perforatum*, HH: *H. heterophyllum*, HS: *H. scabrum*).

Table 2: Repeated measures ANOVA parameters for main effects and associated interactions leading to mortality rates of the three stored-product insects tested (for *R. dominica*, *T. confusum* and *S. oryzae*).

Source	*df*	Species of tested insects					
		R. dominica		*T. confusum*		*S. oryzae*	
		F	P	F	P	F	P
Hypericum specie	2	4.76	<0.01**	21.33	<0.01**	5.77	<0.01**
Plant part	2	29.90	<0.01**	0.06	0.94	2.73	0.06
Exposure time	2	23.90	<0.01**	9.57	<0.01**	15.45	<0.01**
Hypericum specie x Plant part	4	38.96	<0.01**	2.28	0.06	2.63	0.04*
Hypericum specie x Exposure time	4	0.40	0.81	0.27	0.89	0.36	0.84
Plant part x Exposure time	4	0.39	0.82	0.18	0.95	0.02	0.99
Hypericum specie x Plant part x Exposure time	8	0.52	0.84	0.8	0.63	0.38	0.93
Error	135						

p<0.01. **; *p*<0.05.*

Mortality of *T. confusum* treated with *Hypericum* extracts

Mortality of *T. confusum* adults was significantly affected by the *Hypericum* species and exposure time which were found statistically significant effect at P < 0.01. The other main factor (plant part) and all interactions were no statistically significant (P > 0.05) (Table 2).

Among the *Hypericum* species, *H. scabrum* (29.2%) and *H. perforatum* (22.4%) were found to be more effective against *T. confusum*, and there was no statistically significant difference between them (Table 4). Mortality rate was recorded to increase with the exposure time of application in all extracts (Table 4). After 72 h of exposure, the flower of *H. scabrum* had the highest mortality with 42.1% on this pest.

Mortality of *S. oryzae* treated with *Hypericum* extracts

Hypericum specie and exposure time were statistically found to be effective on the mortality of *S. oryzae* (P <0.01) whereas plant part were no significant (P > 0.06) (Table 2). Similarly, there was no different to all interactions except *Hypericum* specie x plant part interaction (F = 2.63, p = 0.04).

The mortality of *S. oryzae* increased with exposure up to only 48 h with each extract application, and there was no statistically significant difference between 48 h and 72 h exposures (Table 5). Among *Hypericum* species, *H. scabrum* (14%) had the highest mortality against *S. oryzae*, but statistically similar as compared to *H. perforatum* (13.0%). After 72 h of exposure, the stem of *H. scabrum* was exhibited the highest mortality with 19.3%.

Table 3: Mean mortality (%±SE) of *R. dominica* adults after 24, 48 and 72 h of exposure on wheat treated with different solvent extracts of the *Hypericum* species.

		Exposure						Average	Specie average
		24h		48h		72h			
Control		0.0	c	0.0	e	0.0	d	0.0 e	
H. perforatum	Flower	0.9±0.93	c	5.1±1.14	de	19.1±1.41	c	8.4 d	42.3 a
	Leaf	76.8±0.06	a	93.2±1.62	a	94.1±1.42	a	88.0 a	
	Stem	27.9±0.20	b	31.0±0.48	bc	32.8±0.42	bc	30.6 bc	
Average		35.2		43.1		48.7			
H. heterophyllum	Flower	20.9±0.27	b	30.8±0.40	bc	30.8±0.40	bc	27.5 bc	31.0 b
	Leaf	16.0±1.18	b	24.3±1.26	bc	31.2±0.26	bc	23.8 c	
	Stem	29.0±1.43	b	48.2±0.17	b	50.2±0.66	b	42.5 b	
Average		22.0 b		34.4 ab		36.7 a			
H. scabrum	Flower	22.7±0.25	b	35.4±0.73	bc	48.1±0.55	b	35.4 bc	31.4 b
	Leaf	20.7±1.33	b	34.2±0.80	bc	48.5±0.69	b	34.5 bc	
	Stem	18.0±1.02	b	19.3±0.22	cd	35.7±0.57	bc	24.3 c	
Average		20.5 b		29.6 b		44.1 a			
Exposure average		25.9 b		35.7 ab		43.2 a			

± SE: standard error. Means followed by the different letters in each exposure intervals are significantly different (Duncan test at 0.05).

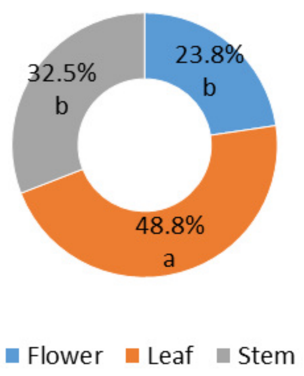

Figure 2: Toxicity rate of plant parts on the mortality of *R. dominica.*

To our knowledge, this is the first work that has examined the insecticidal properties of the *Hypericum* species and their plant parts against *R. dominica*, *T. confusum* and *S. oryzae*. Our results showed that the *Hypericum* extracts caused higher mortality in less time against these insects. But, adult mortality of these insects did not perform similarly and their efficacy depended on exposure time, the *Hypericum* species and plant parts used in the experiment.

The mortality of *R. dominica* and *T. confusum* was found to increase with the exposure of application in all extracts, whereas that of *S. oryzae* increased with exposure up to only 48 h with each extract application.

Hypericum species tested on these insects exhibited strong toxic effect, especially, *H. perforatum* caused the highest mortality to *R. dominica* adults, whereas *H. scabrum* showed the highest mortality for *T. confusum* and *S. oryzae.* But, *H. heterphyllum* exhibited the lowest toxic effect for all insects tested. *H. heterphyllum*, an endemic species to Turkey, were investigated first time toxic effects on these insects, had very little literature about this issue. In an earlier study, dichloromethane extract of *H. heterophyllum* displayed higher inhibiton zone against *Paenibacillus* larvae than that of *H. perforatum* and *H. scabrum*, and other many *Hypericum* species (Hernández-Lõpez et al., 2014). Our results clearly indicated that the efficacy of *Hypericum* extracts varied among the insect species. This may be based on that *H. perforatum* and *H. scabrum* contain higher amount napthodianthrones, phloroglucinols and some flavonoids than *H. heterophyllum* (Smelcerovic et al., 2008; Asan, 2021). In addition, each insect and larva group can react differently to various chemical groups (Zaka et al., 2019).

Table 4: Mean mortality (%±SE) of *T. confusum* adults after 24, 48 and 72 h of exposure on wheat treated with various solvent extracts of the *Hypericum* species.

		Exposure						Average	Specie average
		24h		48h		72h			
Control		0.0	d	0.0	e	0.0	c	0.0 e	
H. perforatum	Flower	15.3±1.72	abc	19.4±1.15	ab	25.4±0.46	ab	20.0 bc	22.4 a
	Leaf	18.6±1.53	ab	20.0±1.78	ab	20.0±1.78	ab	19.5 bc	
	Stem	26.0±0.26	a	27.5±0.32	ab	29.7±0.16	ab	27.7 ab	
Average		20.0		22.3		25.0			
H. heterophyllum	Flower	2.6±0.52	cd	2.6±0.52	cd	12.3±0.67	b	5.8 d	8.5 b
	Leaf	9.0±1.04	abc	9.0±1.04	bc	19.5±1.08	ab	12.5 cd	
	Stem	4.3±0.91	bcd	8.1±1.73	bc	9.2±1.90	b	7.2 d	
Average		5.3		6.6		13.7			
H. scabrum	Flower	30.6±0.48	a	40.4±1.08	a	42.1±1.17	a	37.7 a	29.2 a
	Leaf	17.0±0.77	abc	24.3±0.27	ab	29.1±0.37	ab	23.5 bc	
	Stem	25.2±0.61	a	25.2±0.61	ab	28.8±0.66	ab	26.3 ab	
Average		24.2		30.0		33.3			
Exposure average		16.5 b		19.6 ab		24.0 a			

± SE: standard error. Means followed by the different letters in each exposure intervals are significantly different (Duncan test at 0.05).

Table 5: Mean mortality (%±SE) of *S. oryzae* adults after 24, 48 and 72 h of exposure on wheat treated with various solvent extracts of the *Hypericum* species.

		Exposure						Average	Specie average
		24h		48h		72h			
Control		0.0	c	0.0	c	0.0	c	0.0 e	
H. perforatum	Flower	16.0±0.19	a	16.0±0.19	ab	16.0±0.19	ab	16.0 ab	13.0 a
	Leaf	11.5±0.06	ab	16.4±0.09	ab	16.4±0.09	ab	14.8 ab	
	Stem	5.6±0.62	abc	9.6±0.48	ab	9.6±0.48	ab	8.3 bcd	
Average		11.0		14.0		14.0			
H. heterophyllum	Flower	5.6±0.62	abc	17.0±0.77	ab	17.0±0.77	ab	13.2 abc	7.7 b
	Leaf	2.4±0.95	bc	4.3±0.91	b	4.3±0.91	b	3.7 d	
	Stem	2.2±1.01	bc	8.3±0.39	ab	8.3±0.39	ab	6.3 cd	
Average		3.4		9.9		9.9			
H. scabrum	Flower	9.3±0.95	ab	15.3±0.79	ab	15.3±0.79	ab	13.3 abc	14.0 a
	Leaf	3.0±1.23	abc	15.0±0.89	ab	15.0±0.89	ab	11.0 abc	
	Stem	14.3±0.20	ab	19.3±0.22	a	19.3±0.22	a	17.6 a	
Average		8.9		16.5		16.5			
Exposure average		7.8 b		13.5 a		13.5 a			

± SE: standard error. Means followed by the different letters in each exposure intervals are significantly different (Duncan test at 0.05).

The plant part, one of the main factors, found to be statistically effective only on *R. dominica*. The leaf shown the higher mortality than the stem and flower against this insect. Sun et al. (2019) reported that leaf part of *H. perforatum* at the bloom stage contained higher amounts of polyphenols and flavanoids than its flower part. Çirak et al. (2016) recorded that chlorogenic acid, neochlorogenic acid, avicularin and 2,4-dihydroxybenzoic acid in leaf of eight *Hypericum* species were higher than the other parts. This shows that these chemical compounds or compound groups can act on *R. dominica*. Also, this may depend on the solvent difference or the location, collection time and climatic conditions of plant species. Because type, amount of phytochemicals or their percentages in plant and its parts are affected by these parameters (Nogueira et al., 2008; Sun et al., 2019; Saddiqe et al., 2020). In a previous study, Dastagir, Ahmed and Shereen (2016) notified that none of leaf, flower and fruit extracts with methanol of *H. perforatum* showed effect on mortality of *S. oryzae*, *R. dominica*, *Tribolium castaneum*, *Trogoderma granarium*, *Callosobruchus analis*.

Among tested insects, *R. dominica* was the most sensitive to *Hypericum* extracts, followed by *T. confusum* and *S. oryzae,* respectively. In studies conducted by different researchers, it has been reported that these stored grain insects show different sensitivity to products of various plant and synthetic. Similar results are reported for several pyrrole derivatives against adults of these three stored-product insects (Boukouvala et al., 2019). Bhavya et al. (2019) notified that *R. dominica* was the more susceptible than *S. oryzae* to all of 16 botanical extracts. This difference between insects versus the same product may be related to the insect's genetic.

CONCLUSIONS

Flower, leaf and stem extracts derived from *Hypericum perforatum*, *Hypericum scabrum* and *Hypericum heterophyllum* has high toxic effect for the control of the stored product insects tested here, but this effect varies according to the target species. Especially, the results showed that the leaf extract of *H. perforatum* can be useful in controlling *R. dominica* populations. Because it has a very strong effect against this insect (>94.0%).The stem and flower extracts of *H. scabrum* can be more effect in controlling *S. oryzae* and *T. confusum*, respectively. But the highest mortality rate of *S. oryzae* and *T. confusum* is less than 50%. The main reason for this difference on insecticidal activity in *Hypericum* species and their plant parts is based on its chemical content. Further research is needed to shed light to these different parts of the *Hypericum* species, including phytochemical components of these effective *Hypericum* extracts, and to evaluate the basis of their use in realistic scenarios in stored-product protection.

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