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Microencapsulated essential oils active against indianmeal moth

G. SANNA PASSINO, E. BAZZONI, M. D. L. MORETTI

The toxic effects of *Rosmarinus officinalis* and *Thymus vulgaris* microencapsulated essential oils were assayed against *Plodia interpunctella* larvae. The activity of the oil was observed after diet contamination with the microcapsules and vapours exposition. The effect of the oils is mainly produced by the contamination of the diet with the active principles released from the microcapsules and is a consequence of ingestion. *Thymus* and *Rosmarinus* oils were extremely effective against I-II instar larvae, that at low concentrations (0.1%) gave mortalities over 50%. By increasing the microcapsules concentration in the diet, proportional increases in mortality were recorded, which reached values up to 80% in both treatments. The difference found in the release pattern of the oils could be due to the different hydrophilic characteristics. *Thymus* microcapsules still contains about 75% of the oil after 25 days while *Rosmarinus* formulation only 25%.

G. SANNA PASSINO: Istituto per lo Studio degli Ecosistemi (ISE, CNR) – Sez. Ecologia Applicata e Controllo Biologico, via De Nicola, 1 - 07100 Sassari, Italy (e-mail: gsannapassino@yahoo.it)
E. BAZZONI, M. D. L. MORETTI: Dipartimento di Scienze del Farmaco, via Muroni 23/A - 07100 Sassari, Italy

Key words: *Thymus*, *Rosmarinus*, essential oils, formulations, *Plodia interpunctella*

INTRODUCTION

Synthetic pesticides have been considered until now the only effective means available for the control of stored product pest insects. However, health authorities are rather reluctant to accept chemical residues in food because of adverse effects on humans and the environment (MATSUMURA, 1980). A variety of superior plants may provide new sources of natural pesticides (GRAINGE and AHMED, 1988). The essential oils are considered a powerful source of natural derivatives useful against stored product pests and their insecticidal activities are manifold: fumigant and topical toxicity, ovicidal activity, antifeedant and repellent effects (HUANG *et al.*, 2000; LALE *et al.*, 2000; PADIN *et al.*, 2000; TUNC *et al.*, 2000; BOUDA *et al.*, 2001; LEE *et al.*, 2001). Interesting results have been obtained using encapsulated formulations of the essential oils components (CLANCY *et al.*, 1992). ALLOTEY and GOSWAMI (1994) showed that *Plodia interpunctella* (Hubner) could be controlled using local plant materials. *Plodia interpunctella,* the Indian meal moth, develops on a variety of grains, beans, nuts, and processed foods and it is a major problem during processing and storage (JOHNSON *et al.*, 1992).

The purpose of this work was to test, under laboratory conditions, the effects of *Rosmarinus officinalis* L. (Lamiaceae) and *Thymus vulgaris* L. (Lamiaceae) essential oils formulated as microcapsules, on *Plodia interpunctella* larvae. The essential oil formulations, prepared by a phase separation process (coacervation), were characterized in terms of oil content and composition, size distribution, and release profile.

MATERIALS AND METHODS

Essential oils microencapsulation

Thymus vulgaris and *Rosmarinus officinalis* essential oils (Cruciani, Roma) were used after purification by steam distillation. Their composition was determined by Gas Chromatography (GC) and Mass spectra (GC/MS) analyses using a Carlo Erba HRGC 5300 Mega series gas chromatograph with FID detector and a Hewlett Packard 5890 gas-chromatograph directly coupled to a Hewlett Packard HP 5971A mass selective detector (MSD). Component identification was carried out by comparing the obtained MS data with those reported in Library Wiley on MS-ChemStation HP v. C.00.07. The quantitative data reported are the mean of three analyses. The analysis conditions are described in the literature (MORETTI *et al.* 1998).

The microencapsulation process was carried out by coacervation coupled with a freeze-drying method (MORETTI *et al.*, 2000). An aqueous dispersion containing 10% of gelatine in purified water was prepared at 40 °C using a glass vessel apparatus with an external jacket connected to a circulating thermostatic bath. The essential oil was emulsified using a gelatine/oil ratio of 2 and a high shear mixer (turbine). The coacervate phase was obtained by addition of a suitable amount of Na_2SO_4 (as 20% w/w water solution), and cooling the system to 5 °C, stirring for 1 hour. Glutaraldehyde solution (1 mmol/g gelatine) was then added and pH was regulated to 8 by addition of a suitable amount of NaOH 1N. The resulting mixture was maintained at 5 °C and 750 rpm for 3 hours. The hardened microparticles were filtered, rinsed with cold water and finally dehydrated by freeze-drying. The filtered aqueous phase was collected and transferred into the steam distiller to determine the amount of non-entrapped oil. The encapsulation yield (EY) was determined using the following formula: EY (%) = (C - N/ C) x 100 where C = amount of loaded essential oil (g) and N = amount of non-entrapped essential oil (g). Empty microcapsules, used as a control in the biological assays, were prepared by removing the essential oil by steam distillation from the wet products obtained at the end of each coacervation process. The essential oil content was determined by extracting the encapsulated oil by steam distillation from an accurately weighed amount of dried microcapsules.

Particle size distribution was determined in a LS100 particle size analyzer (Coulter Corporation, Miami, Florida). Analyses were carried out using aqueous dispersions of full and empty microparticles in a suitable concentration. Data are reported as both the mean diameter and distribution expressed by d_{10}, d_{50} and d_{90}, where d_{50} represents the median value of the microparticles size distribution. The dry residue was determined by drying and weighing the empty microparticles using a thermo-gravimetric balance (Mettler LP 15, Swiss) at 105°C.

The release profile of encapsulated essential oils was evaluated at 25 ±1°C and different r. h. (45, 75, 95 ± 5%) using samples containing 2 g of the microparticles placed in 90 mm diameter glass petri dishes, covered with a nylon net, in drying chambers with a K-iodure saturated solution. At set time intervals (1, 3, 7, 11, 15, 25 days) the amount and composition of the residual content of encapsulated essential oil was determined, as previously described. Each experiment was replicated four times and values reported as cumulative release profiles (%).

Bioassays

P. interpunctella was reared in the laboratory at 27±1°C, 60±5% r h, 16:8 h light:dark and placed in plastic jars containing biological maize flour.

Ingestion tests was carried out in 35 mm petri dishes containing groups of 10 larvae at different stages of development (I-II, III-IV instar) and 1 g of a maize flour mixture with different amounts of encapsulated *Thymus* or *Rosmarinus* essential oil (from 0.01 to 4 % w/w of total weight). Larval mortality was recorded after 7 days. Lethal concentration

(LC_{50}) was calculated by the Trimmed Spearman-Karber method (HAMILTON *et al.*, 1977).

Inhalation test was performed using plastic test tubes (height 10 cm; diameter 3.5 cm) closed at the base with a nylon net of tightened meshes. 2 g of maize flour and groups of 10 larvae of II-III instar were placed inside each tube. The test tubes were inserted in the top of glass tubes (height 20 cm; diameter 3.7 cm) containing a selected quantity of encapsulated oil (1.0, 2.5 and 5.0 % w/w with respect to maize flour amount). The experimental apparatus was designed in order to obtain a maize flour phase of 1 cm thickness, kept 10 cm away from the oil formulation. After 7 days, the maize flour was examined to assess larvae mortality and was submitted to repeated extraction with n-hexane to determine its essential oil content.

Each test was replicated 4 times using empty microparticles as a control.

A one-way analysis of variance was performed on transformed data (arcsine of the square root); treatment mean values were separated from those of the control using Student-Newmann-Keuls test. Results of all statistical tests were considered significant if $P<0.05$.

RESULTS

Main components of the essential oils and characteristics of microcapsules

Table 1 shows the chemical composition of *Thymus* and *Rosmarinus* oils, the encapsulation yield, the percentage of humidity and the mean diameter of the microcapsules. The essential oil of *Rosmarinus* contains an elevated percentage of poor polar compounds (monoterpenic hydrocarbons 59%), in particular a-pinene (about 39%). Among the polar compounds we record the presence of 1,8 cineole (8.6%). The essential oil of *Thymus* contains an elevated percentage of phenolic polar compounds (55.5%), among which thymol prevails (49%). The hydrocarbon fraction, instead, represents about 27% (p-cymene 18.5%).

Table 1.- **Composition and characteristics of essential oil microcapsules**

Main components	Thymus vulgaris	Rosmarinus officinalis
	(% w/w)	
α-pinene	0.8	38.7
α-phellandrene	n.d.	0.6
α-thujone	1.3	n.d.
α-terpynene	0.9	n.d.
γ-terpynene	n.d.	0.4
α-pinene	0.6	1.1
α-caryophyllene	2.5	0.1
α-myrcene	1.7	4.5
borneol	n.d.	7.0
bornyl acetate	n.d.	8.3
camphene	0.4	7.8
camphor	n.d.	6.8
carvacrol	6.5	n.d.
1, 8 cineole	n.d.	8.6
geraniol	n.d.	0.1
limonene	1.4	4.2
linalool	2.1	0.9
verbenon	n.d	2.9
p-cymene	18.5	1.5
thymol	49.0	n.d.
Encapsulation yield (%)	98.6	99.2
Essential oil content (%w/w)*	66.5	68.4
Humidity (%)	70.3	60.3
Particle size (μm)		
d10	12.6	13.2
d50	48.9	52.0
d90	116.5	123.9
mean diameter (μm)	59.7	63.5

n.d. not detected
*) Referred to the amount of oil charged

The process used gave a high encapsulation yield (over 98%) with both *Rosmarinus* and *Thymus* oils, and did not cause any appreciable modifications in the chemical composition of the oils. The essential oil contained in the microparticles ranged from 68% (*Rosmarinus* oil) to 66% (*Thymus* oil). The phase separation process used produced particles sized from 12 to 123 mm with an average diameter of about 60 μm. The water

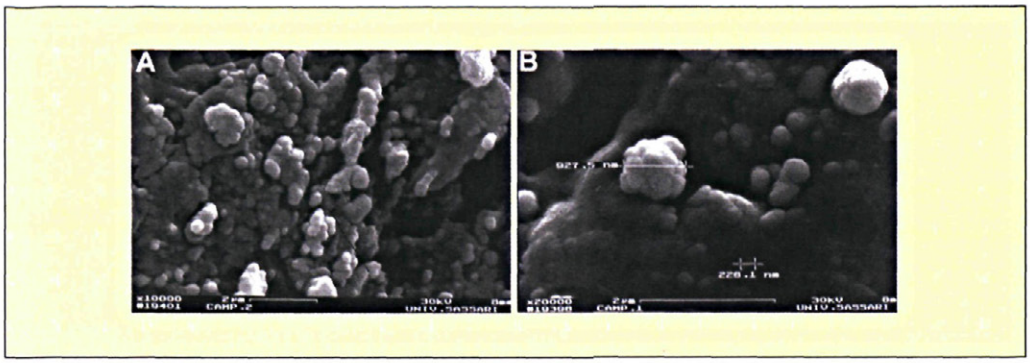

Figure 1: Scanning electron micrographs of microcapsules containing essential oil.

content in microcapsules (% of humidity) was 70% for *Thymus* and 60% for *Rosmarinus*: this indicates that a different entrapment of aqueous phase into the microparticles happens during coacervation.

Microcapsules appeared to be made up of single spherical units of about 0.2 mm diameter, stuck to each other to form a blackberry-like structure. The external surface of each unit was almost regular and smooth (Figure 1).

Release profile of microcapsules

The patterns of essential oils release from the microcapsules are shown in Figures 2 and 3. The greater accumulative release of *Rosmarinus* oil can be evidenced (approximately 75% of the total after 25 days). This was likely due to the greater amount of hydrocarbon compounds. These poor polar compounds are caught in smaller scale by the water contained in the microcapsules and they are quickly released; to the contrary, we

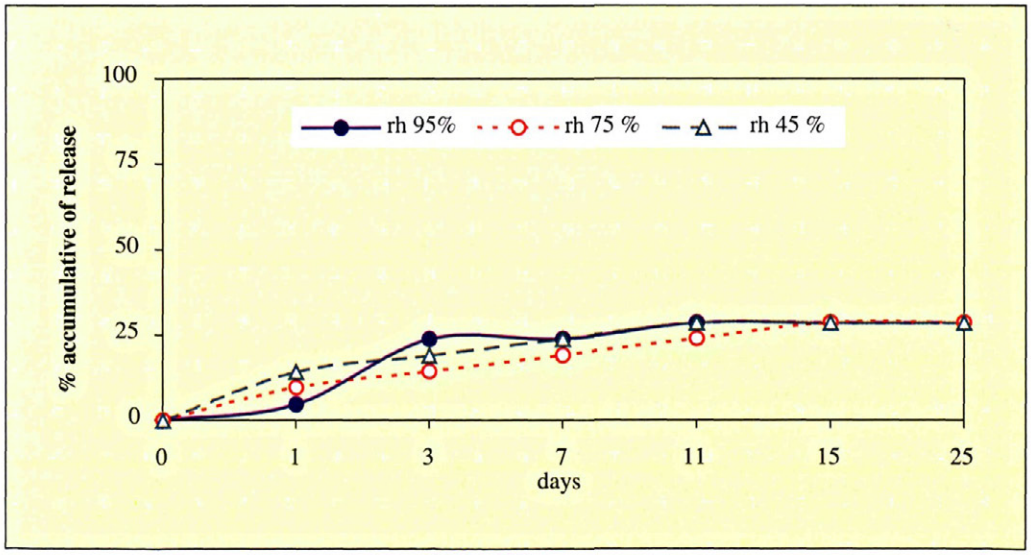

Figure 2: Percentage of essential oil release from *Thymus* microcapsules

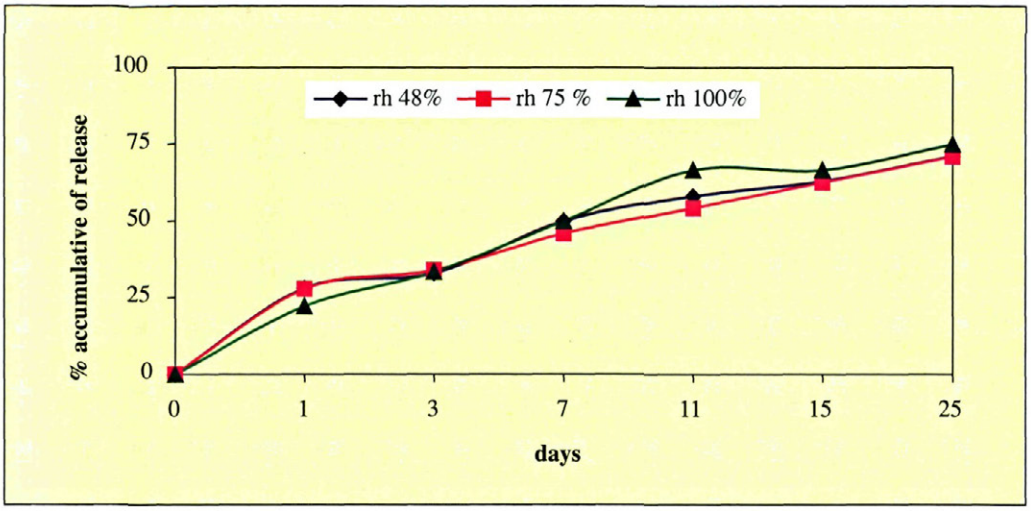

Figure 3: Percentage of essential oil release from *Rosmarinus* microcapsules

have recorded a slower release of *Thymus* oil (approximately 25% in total after 25 days), that instead is rich of phenolic polar compounds.

Toxicity tests

Tables 2 and 3 show the mortality percentages of *P. interpunctella* larvae fed on maize flour containing *Thymus* and *Rosmarinus* oil microcapsules and the multiple range tests from untransformed mortality by essential oil formulations and larval instar. I-II instar larvae showed higher sensitivity than III-IV ones: mortality values, from about 32% to 87%, were obtained with both *Thymus* and *Rosmarinus* formulations with smaller larvae, while the corresponding values, with larger larvae, varied from about 7% to 50%. LC_{50} values were 1.3 mg/g for *Thymus* microcapsules and 2.1 mg/g for *Rosmarinus* microcapsules with I-II instar larvae; the corresponding values obtained with III-IV instar larvae were 83.5 mg/g and 141 mg/g respectively. Table 4 shows the percentage of mortalities of *P. interpunctella* II-III instar larvae exposed for a period of 7 days to vapours of the microcapsules. *Rosmarinus* gave mortalities from 5 to 17% while *Thymus* produced mortalities from 7 to 20%. No significant differences were

Table 2.-**Mean mortality of P. interpunctella larvae fed on maize flour containing Rosmarinus and Thymus essential oils microcapsules (test time 7 days).**

% of microcapsules mixed in the diet	instar	0.01	0.1	1	2.0	4.0
			Mortality (% ± s.e.)			
R. officinalis	I-II	32.5±1.5	52.5±0.8	62.5±1.5	75±0.9	87.5±0.8
T. vulgaris	I-II	35±0.9	55±0.9	67.5±0.8	75±0.9	82.5±1.5
R. officinalis	III-IV	7.5±0.8	15.0±0.9	32.5±0.8	35.0±0.9	40.0±1.3
T. vulgaris	III-IV	12.5±0.8	22.5±1.5	30±1.8	35±0.9	50±1.3
control	I-II	7.5±0.8				
control	III-IV	7.5±1.5				

Table 3: **Multiple range tests from untransformed mortality by essential oil formulations and larval instar. Data referred to ingestion test.**

Essential oil formulations	n*	Mortality mean (%)
R. officinalis microcapsules	40	44.0b
T. vulgaris microcapsules	40	46.5b
control	8	7.5a
Larval Instar		
III-IV	20	28.0a
I-II	20	62.5b

The same letter indicate an absence of statistical difference ($p<0.05$, Student-Newman-Keuls tests)
*groups of 10 larvae

Table 4.-**Mean mortality of P. interpunctella larvae exposed for 7 days at vapours of microencapsulated essential oils.**

% of microcapsules withrespect to the diet	1.0	2.5	5.0
	Mortality (% ± s.e.)		
R. officinalis microcapsules	5.0±0.9	12.5±0.8	17.5±1.5
T. vulgaris microcapsules	7.5±0.8	15.0±1.3	20.0±0.9
control			0.0

found between the treatments above to 2.5 and 5.0 % (Table 5).

DISCUSSION

Thymus and *Rosmarinus* oils proved extremely effective against young larvae (I-II instar), with mortalities at low concentrations up to 50%. By increasing the microcapsules concentration in the diet we recorded proportional increases in mortality of up to 80% in both treatments. From the tests carried out it appears that the toxic effect of exposure to microcapsules vapours is poor. This confirms that the activity of the oils is mainly produced by contaminating the diet with the active principles released from the microcapsules and is a consequence of ingestion. The absence of statistical differences between the two treatments, carried out with formulations containing essential oils of different chemical composition, does not allow us to correlate the toxic activity to none of the single component of the oils. However, the toxicity of 1,8 cineole, an active principle isolated from *Artemisa annua* essential oil, is known (TRIPATHI *et al.*, 2001), and so is the insecticidal and acaricidal activity of thymol (EL GENGAIHI *et al.*, 1996; KARPOUHTSIS *et al.*, 1998; IMDORF *et al.*, 1999). It can be asserted that the recorded toxic effect of each treatment is related to the synergetic action of the single components of the essential oil, as observed in recent studies (BEKELE and HASSANALI, 2001).

Table 5: **Analysis of variance for mortality and multiple range tests. Data referred to inhalation test.**

Main effects	Sum of squares	Df	Mean square	F-ratio	P-value
Essential oil formulations	78.90	1	78.90	2.03	0.20
replications	365.62	3	121.87	3.14	0.10
concentration	782.73	2	391.36	10.08	0.01
Total (corrected)	2164.2	23			

Student-Newman-Keuls tests for mortality by concentrations

% of microcapsules	n*	Mortality mean (%)
1.0	8	6.25a
2.5	8	18.75b
5.0	8	18.75b

The same letter indicate an absence of statistical difference ($p<0.05$)
* groups of 10 larvae

The methods used in the preparation process allowed the essential oils to be entrapped without any changes in their composition. The differences found in the release patterns could be due to the different hydrophilic characteristics of the examined oils. In fact, the high content of polar compounds in *Thymus* oil seems to favour the entrapment of aqueous phase into the microparticles during coacervation and subsequently a slower release.

On the contrary, the greater content of little polar compounds, present in the essential *Rosmarinus* oil, could favour a more rapid release. This effect appears evident if the different amount of essential oil, content in the microcapsules of *Thymus* and *Rosmarinus*, at the end of the test, is considered. This result suggests different applications of these formulations in the integrated pest control strategies, in particular in function of the chemical composition of the essential oils considered.

ABSTRACT

SANNA PASSINO G., E. BAZZONI, M. D. L. MORETTI. 2004. Aceites esenciales microcapsulados activos contra polilla indianmeal. *Bol. San. Veg. Plagas*, **30**: 125-132.

Los efectos tóxicos de los aceites esenciales microcapsulados de *Rosmarinus officinalis* y de *Thymus vulgaris* fueron estudiados contra larvas de *Plodia interpunctella*. La actividad de aceite fue observada después de la contaminación de la dieta con las microcápsulas y después de la exposición a los vapores. La actividad de los aceites es producida, principalmente, por la contaminación con los principios activos lanzados de las microcápsulas y es, por lo tanto, una consecuencia de la ingestión. Los aceites de *Thymus* y de *Rosmarinus* fueron extremadamente eficaces contra los estadios larvarios I y II, determinándose una mortalidad superior al 50% (concentraciones de microcapsulados 0.1%). Aumentando la concentración de las microcápsulas en la dieta, se encontraron aumentos proporcionales en la mortalidad, que alcanzó valores del 80% en ambos tratamientos. La diferencia encontrada en la liberacion de los aceites podía ser debida a las características hidrofílicas. Las microcápsulas de *Thymus* contienen todavia el 75% del aceite después de 25 días mientras que la formulación de *Rosmarinus* solamente 25%

Palabras clave: *Thymus*, *Rosmarinus*, aceites esenciales, formulacion, *Plodia interpunctella*

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