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Article

Selective biorational treatments for managing the storage mites, *Tyrophagus putrescentiae* (Schrank) and *Aleuroglyphus ovatus* (Troupeau) under laboratory conditions

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Abstract

Mites have lately emerged as economically important pests of stored products. Recently, addition of natural origin compounds individually or as a combination with predators have provided a considerable value for controlling these pests. In this study, the efficacy of the bacterium-derived pesticides, spinosad and spinetoram, and the combination of each of them with the predator *Cheyletus malaccensis* Oudemans was evaluated against two storage mite pests, *Tyrophagus putrescentiae* (Schrank) and *Aleuroglyphus ovatus* (Troupeau) under optimal abiotic conditions for pest development. After 21d, the terminal density was estimated for both astigmatid mite species exposed to diet (experiment I) treated with either spinosad or spinetoram (concentrations range of 0.01-2 ppm). Estimation was also done with diet (experiment II) treated with either spinosad or spinetoram (0.5 ppm) and/or the predator at initial predator/prey ratio (0.02). The density of predator was also determined after 21 days. Application of spinosyns significantly reduced population of *T. putrescentiae* and *A. ovatus*. The reduction potential increased with increasing concentration. Complete control of *T. putrescentiae* and *A. ovatus* was achieved by the application of spinosad at 1 and 2 ppm, respectively. As measured by rC_{50} and rC_{90} (concentration for 50% and 90% suppress of population in comparison to control), spinosad was more toxic to *T. putrescentiae* and *A. ovatus* than spinetoram. Furthermore, *T. putrescentiae* was more susceptible to spinosad than *A. ovatus.* Conversely, it was less susceptible to spinetoram than *A. ovatus*. The populations of both mite species were successfully suppressed by the sole application of *C. malaccensis*. Although the density of predatory mites was not affected by the presence of 0.5 ppm spinosad, it was almost eradicated by spinetoram at 0.5 ppm. A combination of spinosad at 0.5 ppm with two individuals of *C. malaccensis* mites (ratio 0.02) outperformed spinosad used alone at the same former concentration in reduction efficiency of the pest populations by 12% for *T. putrescentiae* and 25% for *A. ovatus* within 21 days.

Keywords: Biorational control**,** spinosyns, storage mites, predator, efficacy assessment

Introduction

Mites are regarded as a major pest of stored commodities. Although they are small in size, their numbers can build up rapidly, especially when the infested material is damp enough, which in turn is reflected as reduced quality of the stored material. Storage mites can affect the product's quality directly via damage through feeding (Parkinson 1990) and indirectly via disseminating bacteria and toxigenic fungi (Franzolin *et al*. 1999; Hubert *et al*. 2004). Also, they give rise to many allergic reactions in humans (Kondreddi *et al*. 2006; Fernandez-Caldas *et al*. 2008). Acquiring knowledge about mite species, their distribution and prevalence in specific area is considered an essential step in designing effective management programs. In Egypt, acarid mites accompanied by their predators

from the family Cheyletidae were the most prevailing group of mites in grain stores and markets (Zaher *et al*. 1986; Bakr 2000, 2006).

Conventional chemicals, fumigants and grain protectants are commonly used to control mite pests in storage facilities (Stables 1980; Bowley & Bell 1981; Nayak 2006a). Although effective, quick–acting and easy applicable, some of these have severe restrictions due to safety and environmental concerns (Collins 2006). Another limiting factor is that mites develop resistance to some particular chemicals (Stables, 1984; Szlendak *et al*., 2000). The invention of new insecticide compounds offers new opportunities in controlling stored-product insects (Reeck *et al*. 1997). Among these compounds, the spinosyns play an important role (Dripps *et al*. 2008, Hertlein *et al*. 2011, Vassilakos *et al*. 2012). Spinosad and spinetoram are two such products that represent spinosyn compounds. Spinosad and spinetoram are neurotoxin insecticides that stimulate the nicotinic acetylcholine receptors while antagonizing gamma amino butyric acid (GABA) receptor sites. Both of them are toxic to pests through contact or ingestion, and they give excellent control to numerous key stored product pests (Nayak *et al*. 2005; Athanassiou *et al*. 2009; Vayias *et al*. 2010; Vassilakos *et al*. 2012; Athanassiou & Kavallieratos 2014).

Owing to their origin from the fermentation of the soil actinomycete, *Saccharo- polyspora spinosa* Mertz and Yoa, spinosad and spinetoram are regarded as reduced-risk pesticides with low mammalian toxicity and sound environmental profile (Bret *et al*. 1997; Clevelan *et al*. 2001; Dripps *et al*. 2008). The miticidal activity of spinosad and spinetoram has been documented against phytophagous mites (Bret *et al*. 1997, Vanleeuwen *et al*. 2005; Villaneva & Walgenbach 2006; El Kady *et al*. 2007; Wang *et al.* 2016). However, few reports have addressed the effect of spinosad against the storage mite, *Tyrophagus putrescentiae* (Schrank) (Sánchez-ramos and Castañera, 2003; Nayak 2006a, 2006b). On the other hand, spinosad is minimally toxic against the predators of stored-product insects (Toews & Subramanyan 2004; Parker & Falconer 2007), whereas, it was recorded by Lefebvre *et al.* (2011); Beers and Schmidit (2014) and by Kim *et al.* (2018) that spinetoram caused mortality to predatory phytoseiid mites. However, to the best of our knowledge, both compounds have not yet been tested against the predators of stored-product mites.

Predators of the family Cheyletidae, such as *Cheyletus eruditus* (Schrank) *and C. malaccensis* Oudemans occur naturally in stored products (Žďárková 1979). *Cheyletus malaccensis* is an oligophagous predator of Acari and has been used for the biocontrol of storage mite pests (Pekar & Hubert 2008; Cebolla *et al*. 2009). However, biocontrol alone by predatory mites sometimes fails to suppress high pest infestation (Žďárková 1998). Because of that, the management strategy of using predators in combination with other treatments like spinosyns, if these compounds are toxic to pest mites and not to the predators, may increase the success of pest mite control.

Therefore, this study was designed to evaluate the individual effect of spinosad, spinetoram and the predator *C. malaccensis* Oudemans on the target pest mites, *T. putrescentiae* and *Aleuroglyphus ovatus* (Troupeau), as well as the possible side effects of the applied compounds on the predator. Moreover, the feasibility of combining the predator with such compounds was tested with the aim of obtaining more efficient control.

Materials and Methods

Mites

Mite specimens used in this study were originally collected from infested samples of stored products obtained from grain stores, Alexandria, Egypt. The mite colonies were grown in the Agricultural Acarology Laboratory (Applied Entomology and Zoology Department, Alexandria University). The astigmatid mites, *T. putrescentiae* and *A. ovatus* were reared in plastic flasks

containing a mixture of bran, flour and dried yeast (40:10:1, wt:wt) (Iatrou *et al*., 2010). The rearing flasks were covered by muslin and kept at 25 ± 1 ^{0}C and 75 ± 5 RH in the dark. The Cheyletid mite *C. malaccensis* was reared by the modified Žďárková (1986) method in paper bags filled with lettuce seeds. Ten females of *C. malaccensis* and 1000 individuals of *A. ovatus* were transferred to the bags. The bags were kept at 25±1 ^{0}C and 75±5 RH in darkness. Cultures of all mite species had been sustained in the laboratory for eighteen months without subjection to any pesticides.

Pesticides

Spinosad (a mixture of 50-95% spinosyn A and 50-5% spinosyn D) was provided by Dow Agrosciences as Tracer® SC (24 g ai /liter) Reg. No.1057 (Cairo, Egypt). Spinetoram (a mixture of the naturally –occurring spinosyn J (major component) and L) was provided by Dow Agrosciences as Radiant® SC (12 ai/liter) Reg. No.1329 (Cairo, Egypt).

Treatments and Bioassay

Clean and infestation free rearing diet (see above) was used for experimentation. Two sets of experiments were conducted.

Experiment I

As a suspension in distilled water, spinosad and spinetoram were homogeneously incorporated into the diet following the method of Nayak and Daglish (2007) to obtain the following concentrations: 0 (Control), 0.01, 0.1, 0.5, 1 and 2 µg g^{-1} diet (ppm). Glass cylindrical vials (4 cm base radius x 3 cm height) were filled with 1 g of the experimental diet. Then, 100 mixed-sex adults of each astigmatid mite species were placed in each vial. Ten replicates per pesticide, concentration and species were covered with a textile mesh and kept at 25 ± 1 ^{0}C and 75 ± 5 RH in darkness.

Experiment II

The median dose (0.5 ppm) of spinosad or spinetoram was used to evaluate its effect on the predator *C. malaccensis,* two large female nymphs of the predator were added along with 0.5 ppm of either spinosad or spinetoram into experimental vials each containing 1 g of diet contaminated with 100 individuals of each of the astigmatid mite species in order to achieve an initial predator-prey density of 0.02.

Diet treated with distilled water only served as a control. The experimental vials were covered and kept as described above. Ten replicates for each treatment combination were used. After 21 days, all experiments were terminated by adding 10 ml of 80% ethanol to each vial (Erban *et al*. 2009). The pest and the predatory mites were directly counted under dissection microscope (Zeiss, Germany) to estimate the population size.

Data analysis

The data were subjected to ANOVA procedure. The doses rC_{50} and rC_{90} for pesticide concentrations causing 50 and 90% decrease of the population after 21 days were estimated for each astigmatid mite species from regression models (Hubert et al., 2007). Means were compared by using the Tukey-Kramer (HSD) test at 0.05 probability level (Sokal & Rohlf 1995). Normality and homoscedasticity of data were checked and augmented by data transformation when needed.

Results

Susceptibility of T. putrescentiae *and* A. ovatus

The results of the analyses data showed highly significant effects of species, pesticide, concentration and associated interactions on the final mite density (Table 1). The final mite population was influenced by the effect of species, as their population growth differed. On control diets, the final density of *T. putrescentiae* was higher than of *A. ovatus* (Fig. 1).

TABLE 1. ANOVA parameters for main effects of species, pesticide and concentration and associated interactions on the final density of *T. putrescentiae* and *A. ovatus.*

	df	F	P
Species	1	223.02	0.0001
Pesticide	1	295.13	0.0001
Concentration	5	131	0.0001
Species X Pesticide	1	206.49	0.0001
Species X Concentration	5	2.76	0.0193
Pesticide X Concentration	5	14.47	0.0001
Species X Concentration X pesticide	5	10.22	0.0001

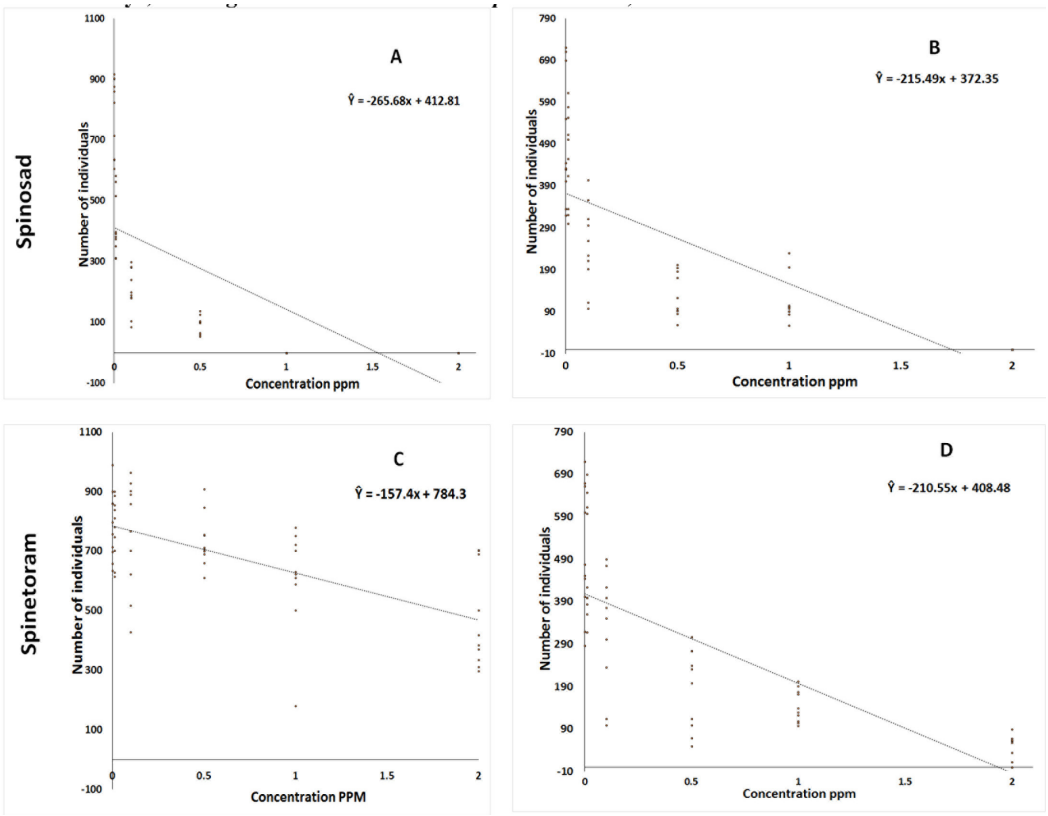

FIGURE 1. The effects of spinoscyn compounds on population development of Astigmatid mites after 21 days, starting with 100 mites. AC—*T. putrescentiae*; BD—*A. ovatus*.

The separate effect of pesticide concentration on the final density of tested mite species is shown in Fig 1. The interaction between pesticide and concentration can be obviously shown from the rC_{50}

and rC_{90} values (Table 2). Comparison of the rC values manifests that spinosad was more toxic to *T. putrescentiae* and *A. ovatus* than spinetoram. In addition, these results indicate that *T. putrescentiae* was more susceptible to spinosad than *A. ovatus*, but, it was less susceptible to spinetoram than *A. ovatus* (Table 2).

TABLE 2. Effect of spinosyn compounds on the final density of *T. putrescentiae* and *A. ovatus* after 21 days.

Pesticide	Mite species	rC values (ppm)		R^2	P
		rC_{50}	rC_{90}		
Spinosad	*T. putrescentiae*	0.072	1.25	0.452	< 0.001
	A. ovatus	0.559	1.494	0.576	< 0.001
Spinetoram	*T. putrescentiae*	2.48	4.482	0.405	< 0.001
	A. ovatus	0.744	1.70	0.569	< 0.001

rC_{50} and rC_{90} ; concentration for 50 and 90% decrease of mite population in comparison to control.

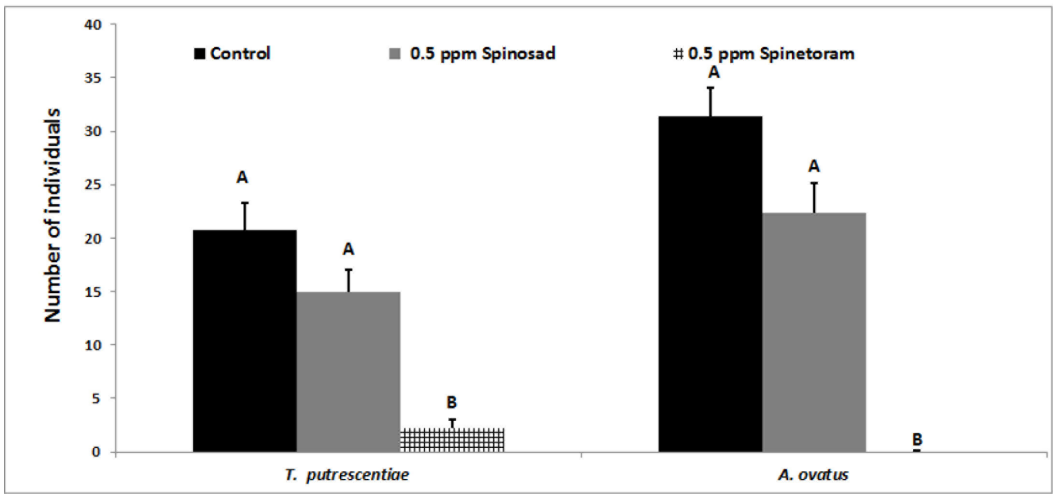

FIGURE 2. Population density (mean ± SE) of *C. malaccensis* reared at 0.02 predator:prey ratio on diet infested with *T. putrescentiae* or *A. ovatus* and treated with 0.5 ppm of the spinosyn compounds after 21 days of exposure. For each species means accompanied by the same letter are not significantly different; HSD test at 5%.

Susceptibility of C. malaccensis

The final density of *C. malaccensis* at an initial predator-prey ratio (0.02) differed significantly with prey species (ANOVA, F= 34.14, P< 0.0001, Fig. 2). It was found to be higher on *A. ovatus* than on *T. putrescentiae.* Although, the density of predator reared on *T. putrescentiae* and *A. ovatus* was not affected significantly by the addition of 0.5 ppm spinosad, it was almost completely eradicated by the application of spinetoram at 0.5 ppm on the diet within 21 days (Fig. 2).

Spinosyns and/or predator potential on astigmatid mites

Comparison of spinosad or spinetoram at 0.5 ppm, *C. malaccensis* at the tested ratio (0.02), and the combination of predator with spinosad at the above mentioned ratio and concentration against *T. putrescentiae* and *A. ovatus* was presented in figures 3A and 3B.

After 21d exposure period, the population growth of *T. putrescentiae* varied significantly among different treatments with the exception of spinetoram at 0.5 ppm in comparison to control (Fig. 3A). The combination of two individuals of predatory mite (i.e., ratio 0.02) and 0.5 ppm spinosad caused complete extinction of *T. putrescentiae* within 21 days (Fig. 3A). In the case of *A. ovatus*, even though, the four treatments reduced the density of the pest mite, the combination of the predator and spinosad had the strongest effect on *A. ovatus* populations (Fig. 3B).

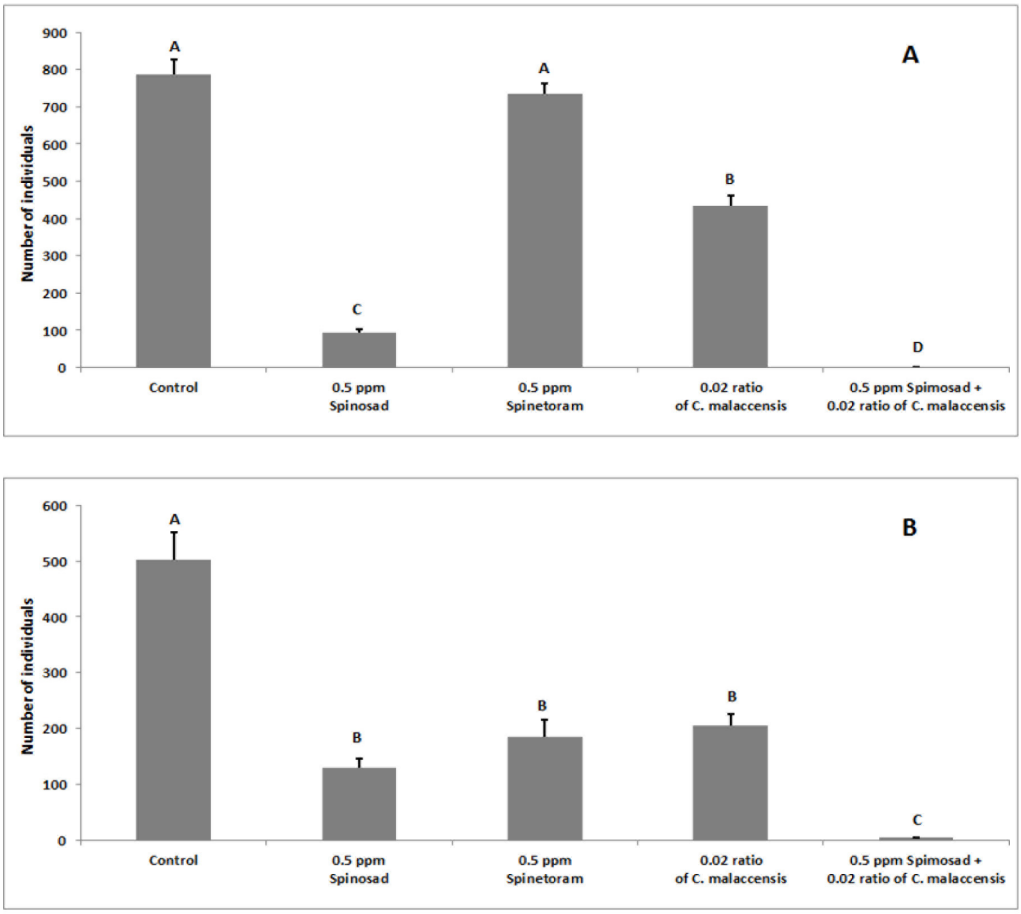

FIGURE 3. Population density (mean ± SE) of *T. putrescentiae* (A) and *A. ovatus* (B) as affected by different treatments for 21 days, means accompanied by the same letter are not significantly different; HSD test at 5%.

Discussion

Available laboratory studies reveal that spinosyn compounds, like spinosad and spinetoram, represent a valuable tool in the finite arsenal of grain protectant products (Hertlein *et al*. 2011; Vassilakos *et al*. 2012; Athanassiou and Kavallieratos 2014). Spinosad and spinetoram provide an effective and long-lasting control against numerous stored product pests at a low rate of 1 ppm (1 mg ai/kg of grain) (Toews and Subramanyam 2003; Nayak *et al*. 2005; Huang *et al*. 2007; Athanassiou *et al*. 2008; Vayias *et al*. 2010).

Although our study confirmed the suppressive effect of spinosyn compounds against the acarid mites *T. putrescentiae* and *A. ovatus*, it did not manifest complete eradication of both species by spinetoram at the tested concentrations. *Tyrophagus putrescentiae* and *A. ovatus* were more susceptible to spinosad as they were fully suppressed by the application of spinosad at 1 and 2 ppm, respectively (Fig. 1). Our findings are consistent with the previous study conducted by Nayak (2006b) which indicated that the mold mite, *T. putrescentiae* was exterminated from wheat treated by 1 ppm spinosad after at least 3 weeks of continuous exposure. In contrast, Sánchez-ramos and Castañera (2003) noted that this species was not effectively controlled by 10.000 ppm spinosad in a diet-incorporation bioassay. These mixed results indicate that a variation in susceptibility among different strains of the same species is likely to occur. Athanassiou *et al*. (2008) reported that considerable differences in sensitivity among different European populations of the confused flour beetle, *Tribolium confusum* Jacquelin du Val to spinosad. These variations in sensitivity levels could possibly happen even among strains from neighboring areas (Kljajc & Peric 2006). This should be taken into account when a controlling strategy is planned.

The formulation type could also have a significant effect on spinosyns' performance (Hertlein *et al*. 2011). A liquid SC spinosad formulation (as the one used in our study) was more effective than the dry formulation when both were applied to stored wheat against *Sitophilus oryzae* L. (Chintzoglou *et al*. 2008).

Results of activity in our study observed that the rC_{50} and rC_{90} were different for *T. putrescentiae* compared with *A. ovatus.* This suggests that both mite species may exhibit biochemical and physiological differences that are probably associated with these variations.

On the other hand, the effect of both pesticides was also studied on the predator *C. malaccansis*. It is possible that the predator population is being reduced either due to direct toxicity of pesticide or indirectly through pesticide's impact on their host species and once their prey population is eradicated, the predator population will come down quickly. Therefore, in order to exclude the indirect effect, the median dose of both pesticides was chosen to evaluate its effect on the predator. Our results show that almost no survivors of *C. malaccansis* were found by the application of spinetoram at 0.5 ppm to the diet. However, *C. malaccansis* population remained unaffected by the application of spinosad at the same above concentration. These results are in harmony with studies performed on other beneficial arthropods, e.g., application of spinosad at 1 ppm to stored wheat or sorghum had no negative effect on the predatory bug, *Xylocoris flavipes* (Reuter) (Toews & Subramanyam 2003; Parker *et al*. 2004). However, the application of spinetoram caused acute motrality to the principals phytoseiid mite predators *Galendromus accidentalis* (Nesbitt) at 1.31 g ai/ L and to *Neoseiulus fallacis* (Garman) with LC_{50} 0.05 g ai/L (Lefebvre *et al*. 2012; Beers & Schmidt 2014).

Spinosyns act on the GABA and nicotinic receptors through mouth or surface contact especially with soft body pests (Athanassiou *et al*. 2008). Symptoms of poisoning in *Tetranychus uriticae* with spinosad were consistent with typical toxicity effects noted with insects: paralysis, refraining from feeding and reduced ovipostion (Van Leeuwen *et al.* 2005).

In our findings, the negligible effect of spinosad on the predator was positively utilized in a combination between spinosad and *C. malaccensis* especially since the predatory mites alone were not recommended to control mite pests with high infestation level (Žďárková 1998). Furthermore, *C. malaccensis* was harmful to human health at high densities (Yoshikawa 1995). On the other hand, obtaining a complete control of tested mite species could be attained using a relatively lower rate of spinosad combined with the predator in contrast to spinosad used independently. So the possibility to reduce costs and total residues increases. Hubert and Pekár (2009) showed that a combination of *Cheyletus* mites and bean flour (as an antifeedant) outperformed bean flour alone in controlling *T. putrescentiae* populations.

On the other hand, assessment of the trophic breadth and prey preferences of a biological control agent is believed to be a substantial step in the study of its potential. Our results show that within three weeks, the population growth of *C. malaccensis* on *A. ovatus* diet surpassed that on *T. putrescentiae* diet with an initial 0.02 predator-to-prey ratio. The current results stand in accordance with the findings reported by Cebolla *et al.* (2009), where it was shown that the rate of population increase of *C. malaccensis* was higher on diets of wheat grain infested with *A. ovatus* than on diets infested with *T. putrescentiae* at predator-to-prey ratio of 0.02 in spite of the fact that *A. ovatus* had lower population density than *T. putrescentiae*. It is possible that *A. ovatus* may have greater nutritional quality for *C. malaccensis* than *T. putrescentiae* promoting higher population growth. The nutrient composition of the prey has a significant effect on growth and survivorship of their predators (Mayntz & Toft 2001).

Spinosyn products evaluated in this study demonstrated a promising potential to be used as alternatives to conventional synthetic compounds, especially, spinosad where it can be effectively integrated into mite management–based program. Further studies are required to discover other biologically active compounds to be used in conjunction with predatory mites naturally coexisting with storage mites in order to obtain broader protection against key mite pests.

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