Abdel-Razek and Abd-Elgawad
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Spinosad combined with entomopathogenic nematode for biocontrol of the Mediterranean fruit fly (*Ceratitis capitata* [Wiedemann]) on citrus

Atef S. Abdel-Razek[1] and Mahfouz M. M. Abd-Elgawad[2*]

Abstract

Background: Cultivation of citrus (Sapindales: Rutaceae) crops is continuously expanding in Egypt given the favorable ingredients of citriculture. Notwithstanding the Egyptian rank as the world's largest orange exporter, the Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the major pests that considerably reduces the quality of citrus crops. Contrary to hazardous organophosphate insecticides that are commonly used to control the Medfly, biologically-based *C. capitata* control tactics were tried herein. The effect of spinosad as a bacterial fermentation product and the nematode *Steinernema riobrave* as biological insecticides applied singly or in combination on laboratory and field strains of Medfly were investigated.

Results: A significant difference in LC_{50} values was observed between laboratory strain (4.78 PPM) and field strain (8.12 PPM) of *C. capitata* larvae exposed to spinosad. A 1.7 fold decrease in susceptibility of field strain was recorded after treatment with spinosad. In a field experiment, a reduction in Medfly population by 80, 37, and 92% for spinosad, *S. riobrave*, and spinosad + nematode treatments was recorded, respectively.

Conclusions: Utilization of spinosad-*S. riobrave* combination in citrus fields, as a novel alternative for unhealthy chemical insecticides to control *C. capitata* in Egypt can be suggested. Use of this combination should be incorporated into a holistic management package that can be economically feasible and environmentally sustainable for Egyptian agriculture.

Keywords: *Ceratitis capitata*, Tracer 24, *Steinernema riobrave*, Insect management

Background

Citrus production suffers from the infestation by many insect pests of which the Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) ranks high. It is one of the most globally damaging pests of horticulture in the fruit fly group. Several specific control methods have been developed and applied successfully in many countries against *C. capitata* (Abd-Elgawad 2021). Common techniques are partial or cover-spraying of chemical insecticides with or without lures, hygiene, the Sterile Insect Technique (SIT), and the Bait Application Technique (BAT). They are recommended for control and suppression of *C. capitata* populations in fruit groves including citrus. Females of Tephritidae flies need certain amino acids as nutrition for developing their eggs and so they are attracted by the relevant baits. Buminal (5, 10 and 15% concentrations) was superior in attracting the Medfly (Amin 2003) as BAT is a widely used approach for general control of fruit flies.

*Correspondence: mahfouzian2000@yahoo.com
[2] Plant Pathology Department, National Research Centre, El-Behooth St., Dokki, Giza 12622, Egypt
Full list of author information is available at the end of the article

Development of chemical insecticides' resistance in pest and vector populations, the damage caused to non-target organisms, and the realization of other environmental hazards of these chemicals have led to an increasing interest in biological control measures (Trdan et al. 2020). Entomopathogenic nematodes (EPNs) are safe biocontrol agents that have been isolated and used successfully in the control of many insect pests in Egypt (Abd-Elgawad 2020) and elsewhere (Koppenhöfer et al. 2020). Moreover, chances that ease incorporating them into holistic management systems of various pests and pathogens should be seized. For example, using EPNs with certain chemical insecticides have proved to increase their effectiveness against pests (Koppenhöfer et al. 2020). Also, developing novel (compatible) techniques or leveraging synergies between EPNs and other pest management tactics to offer economically feasible application could be sought (Abd-Elgawad 2019). Thus, utilizing EPNs + spinosad, a bacterial fermentation product, is hypothesized in the present study to increase their biocontrol efficacy against *C. capitata* populations. As a natural substance, spinosad is toxic to fruit flies either by contact or following ingestion as it contains 2 chemicals: spinosyn A and spinosyn D (Ekesi et al. 2016).

This study examined the following points: (1) basic susceptibility of Medfly to spinosad, (2) susceptibility of Medfly to the EPN *Steinernema riobrave*, and (3) synergistic effects between spinosad and *S. riobrave*.

Methods

Spinosad

Spinosad was obtained from Dow-Agro-sciences as Tracer 24%® formulation. Spinosad has already been organically certified (Racke 2007) and is being used against many insect pests, especially different fruit fly species (Abd-Elgawad 2021).

The entomopathogenic nematode, *Steinernema riobrave*

This highly pathogenic species is maintained in the microbial control laboratory, Giza, Egypt. It was originally isolated from the Rio Grande Valley of Texas, USA, and possesses several promising features (Grewal et al. 2005); its effective host range runs across multiple insect orders. It has ability to exploit aspects of both ambusher and cruiser means of finding hosts and tolerate soil temperatures at approx. 35 °C with persistence even under semi-arid conditions. Its small size provides high yields of EPN-infective juveniles (IJs) whether using in vivo or in vitro production methods.

Laboratory tests

Two different *C. capitata* strains were used, a laboratory strain (L) and a field strain (F), collected from Mashtoul El-Souk Center, Sharkia Governorate, Egypt. The (L) strain was obtained from the standard laboratory culture, Egypt. Medfly was reared on a standard laboratory media; a formulated diet contained 4.83% Nutrifly, 15% corn cob fractions, 8% corn flour, 8.33% sugar, 0.23% sodium benzoate, 0.11% niacin, 0.13% citric acid, and 63.37% water (Hernandez et al. 2010) under constant conditions (25 ± 3 °C and 70–80% RH with a 16:8 (light: dark) cycle. Bioassay of the biological activity of the spinosad (Tracer 24%®) was determined, using treated media bioassay. The first instar larvae of *C. capitata* were used for both *C. capitata* strains. Spinosad doses used in both bioassays were 0 (only water), 2, 4, 8, 16, 32, 64, 125, 250, 500 and 1000 part per million (ppm). All sterile 9-cm-diam. Petriplates of treated diet with spinosad were first air-dried for 1/2 h. Control plates were prepared by diets treated with only distilled water. Afterwards, 5 first instar *C. capitata* larvae of each strain were separately placed in single plates and allowed to feed on the treated media for 24 h. Percentages of mortalities were measured after 24 h. The experiment was repeated 3 times in a completely randomized design.

Field experiment

Three rows each contains 12 'Succari' orange trees of 15 years old were divided into 4 blocks (3 trees each) in an organic farm at Mashtoul El-Souk Center, Sharkia governorate, Egypt. Treatments' blocks were separated by a minimum buffer area of 25 m. Treatments were carried out in a randomized complete block design via applying the following 4 treatments just before sunset in January 2020: (1) Spinosad as cover spray treatment at the rate of 110 ppm in 5 l water tree^{-1}, (2) *S. riobrave* as cover spray at the rate of 3×10^6 IJs in 5 l water tree^{-1}. The spray (EPN suspension) is assumed to fall either on the fruits to protect them from depositing eggs by the insect females and kill the hatched larvae or drain into the ground beneath the tree canopy to kill the emerging insect adults, (3) *S. riobrave*-IJs + spinosad in 5 l water tree^{-1}, and (4) untreated control group (sprayed with water only). Treatments were applied as a foliar and fruit spray, using a back-back sprayer system with a nozzle attached to spray the whole tree. Three plastic International Yellow Pheromone traps with Concept's Medfly Biolure® (ammonium acetate, putrescine, trimethylamine) were placed in the middle of each replicate for the 4 treatments, 5 m between adjacent trees and 35 m between adjacent blocks to attract male flies in addition to the predominant female via attractive integrated pest management (IPM) trap with Biolure® as food bait (Ekesi et al. 2016). Traps were placed just after treatment applications, checked and removed 7

d after application. Data were collected based on the number of the Medfly trapped.

Statistical analysis

Data were analyzed using probit analysis models in the Stat program (Finney 1964; Brown et al. 2001). Mortality rates were corrected by using the Abbott formula (Abbott, 1925). The significant differences between spinosad concentrations expected to kill 50% of *C. capitata* larvae or median lethal concentration (LC_{50}) values based on overlap of 95% confidence intervals were recorded. Data were analyzed using one-way analysis of variance (ANOVA) and followed by the least significant difference (LSD) test as a comparison of the mortality means. Dose–response chart of the dosage were plotted, using percentage mortality rates in Microsoft Excel spreadsheets. A randomized complete block design consisting of 4 treatments, with 3 replicates, each with 3 trees was used. Their field data were analyzed via two-way ANOVA and means separated using Tukey's ($P=0.05$).

Table 1 Percent mortality of two *Ceratitis capitata* strains by spinosad doses

Concentrations No	Concentrations ppm	Percent corrected mortalities	
		Laboratory strain	Field strain
1	2	0	0
2	4	18a	10a
3	8	35a	20a
4	16	40a	22b
5	32	42a	30b
6	64	60a	42b
7	125	70a	62b
8	250	100a	70b
9	500	100a	100a
10	1000	100a	100a

* Mortalities in the same raw followed by different letters are significantly ($P \leq 0.05$) different from each other according to Tukey's test

Results

Laboratory bioassays

The *C. capitata* larvae displayed a concentration-dependent response to spinosad. A 100% mortality of the *C. capitata* field strain was detected after using 250 and 500 ppm but all doses induced mortality of the Laboratory strain ($P<0.05$). An average of approximately 125 ppm of spinosad caused 70 and 62% mortality of laboratory and field strains, respectively (Table 1). According to LC_{50} values (Table 2), a significant difference was observed between laboratory strain (4.78 ppm) and field strain (8.12 ppm), 24 h. after treatment. Therefore, the laboratory strain was more susceptible to spinosad than the field one (Table 2). Obtained results suggested that even common confidence profiles set in this test would not overlap based on the 95% confidence interval (CI). The former strain demonstrated 4.96–4.59 CI at 95% probability level (slope = 2.85 ± 0.001) compared to 9.25-6.99 CI at 95% probability level (slope = 8.09 ± 0.39) for the latter strain whose susceptibility decreased to 1.7 fold.

Field experiment

There was a significant ($P \leq 0.05$) difference between the control and any of the 3 other treatments for the captured Medfly (Table 3). Means of the captured Medfly adults showed that the least number was recorded at the combined treatment of spinosad and nematode followed by spinosad alone, and then nematode alone in an ascending order. The highest numbers of the captured Medfly were recorded in the untreated check.

Table 3 Means of captured Medfly by traps baited in different treatments at 'Succari' orange grove

Treatments	Mean No. of Medfly adults/ Trap*
Spinosad	25.7 ± 6.80[a]
Steinernema riobrave	45.6 ± 3.10[b]
Spinosad + *S. riobrave*	10.12 ± 5.45[c]
Control	125.00 ± 62.75[d]

* Means followed by different letter are significantly ($P \leq 0.05$) different from each other according to Tukey's test

Table 2 Probit analysis for two *Ceratitis capitata* strains treated with different concentrations of spinosad

Strain	Slope ± Standard Error	LC_{50} as ppm (95% CI)[a]	LC_{90} as ppm (95% CI)	Variance	Chi-square	RR[b]
Laboratory	2.853 ± 0.001	4.78 (4.96–4.59)	5.30 (9.45–3.35)	0.000072	0.00098	–
Field	8.09 ± 0.39	8.12 (9.25–6.99)	11.69 (19.8–3.25)	0.00095	0.00	1.7

[a] Confidence interval at 95% probability level; b: Resistance Ratio

These results showed a reduction in Medfly populations by 80, 37 and 92%, for spinosad, nematode, and spinosad + nematode treatments, respectively, compared to the control treatments.

Discussion

In the laboratory study, the LC_{50} values concerning *C. capitata* 1st instar larvae after 24-h exposure to the biological compound, containing concentrate formulation of spinosad, was quite different between the 2 insect strains. Based on these LC_{50} values of spinosad, the field strain was less susceptible than the laboratory strain possibly due to drop in fitness of this latter as a common phenomenon in laboratory-reared organisms based on excessive culturing (Yu et al. 2010). As the laboratory strain has been in culture quite longer than the field strain, it is conceivable that some of the higher mortality recorded in the laboratory strain is due to trait deterioration in the laboratory strain. This goes in parallel to spinosad LC_{50} of *C. capitata* in Tunisia where 22 ppm and 127.95 ppm for laboratory and field population, respectively, were recorded (Guerfali et al. 2020). Reasonably, the field strain may simply have innate fitness factors.

Nevertheless, since the field strain was approximately 1.7 fold more insensitive to spinosad than the laboratory strain, the difference might also be due to the differential susceptibility of strains and intensive insecticide selection pressure in that area of collection. Notwithstanding the above-mentioned innate fitness in field populations of insect pests, a variety of biotic and abiotic factors can contribute to the apparent difference and fitness of field population relative to laboratory one (Yu et al. 2010). Moreover, Stark et al. (2004) found that spinosad was remarkably similar in toxicity to all 3 economically important fruit fly (Diptera: Tephritidae) species: *C. capitata*, the melon fly *Bactrocera cucurbitae* (Coquillett), and the oriental fruit fly *B. dorsalis* (Hendel).

Obtained data proved that spinosad had not only significant effectiveness in the control of *C. capitata* but also its combination with *S. riobrave* could improve the EPN efficacy as biocontrol agent. Therefore, in order to minimize the negative effects of the chemicals on the environment and natural enemies in the management of pests, the natural insecticide spinosad could also be rotated with other pesticides or in low rates simultaneous with different insecticidal categories to avoid development of insect resistance in such programs. On the other hand, the present experiment showed a strong efficacy of the spinosad (*i.e.* 100% mortality) on *C. capitata* when applied at high rates, resulting in complete control in the laboratory bioassays. However, it is expected that the resistance to the spinosad might be developed if it is applied exclusively, i.e., without other biocontrol agent such as entomopathogenic nematodes. Moreover, field spraying of 'Succari' orange trees with a combination of spinosad-nematode material compared to either spinosad alone or *S. riobrave* alone and control provided acceptable control of the pest in commercial citrus cultivations. Likewise, comparable results of spinosad have been observed for wild Medflies in an eradication plan in Guatemala and Mexico (Rendon et al. 2000). Such plans should be fully exploited especially in the new citrus-cultivated areas as a recently published study (Dritsoulas et al. 2021) confirmed that reclaimed desert habitats of Egyptian citrus favor entomopathogenic nematode and microarthropod abundance. On the contrary, an exclusive use of spinosad may possibly generate the insect resistance (Guerfali et al. 2020). This might be indirectly shown herein by the higher resistance in field strain than laboratory strain as well. Therefore, spinosad may be used with a precaution that its long-term use can be contingent.

Conclusions

Obtained results indicated that spinosad + *S. riobrave* together may be used as an effective alternative tool for control of the Medfly in Egypt. As a novel alternative for synthetic chemical insecticides to control *C. capitata* in Egypt, this tactic should be applied on a wider scale to be incorporated into an integrated pest management programs to achieve economically feasible and environmentally sustainable agricultural systems. Additional studies are warranted to determine the economic feasibility of utilizing the combination used herein to control the Medfly.

Abbreviations

SIT: Sterile Insect Technique; BAT: Bait Application Technique; EPNs: Entomopathogenic nematodes; IJs: Infective juveniles; IPM: Integrated pest management; ANOVA: Analysis of variance; CI: Confidence interval; LC_{50}: Median lethal concentration; LSD: Least significant difference; PPM: Part per million.

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Authors' contributions

All authors participated in the development and implementation of the reviewing plan and subsequently written it. The first author AA discussed the different parts of the article with MA and finalized the manuscript. All authors have read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Pests and Plant Protection Department, National Research Centre, Dokki, Giza 12622, Egypt. [2]Plant Pathology Department, National Research Centre, El-Behooth St., Dokki, Giza 12622, Egypt.

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