EVALUATION OF SALIBRO AS A NEW NEMATICIDE FOR COTTON PRODUCTION SYSTEMS

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Abstract

Salibro (Reklemel) was evaluated *in vitro* and *in planta* for its potential inhibitory effects on *Meloidogyne incognita* and *Rotylenchulus reniformis* egg hatch, juvenile (J2) mortality, and subsequent reduction of nematode population density in cotton plants. In both *in vitro* and *in planta* tests, Reklemel was applied at 1, 5, 50, and 250 ppm. Sterile water was added as the control in both tests. Nematode egg hatch and development was measured for nine days whereas J2 mortality was determined for seven days. In greenhouse tests, plant and nematode data were collected at 35 DAP and the data were analyzed with SAS 9.4 using PROC GLIMMIX. The LS-means were compared using Tukey-Kramer's method ($P \leq 0.05$). The result of *in vitro* egg hatch assay indicated that only Reklemel at 250 ppm had an inhibitory effect on egg hatch over 9 days of exposure, but J2s of *M. incognita* were affected within 24 hours of exposure in all concentrations. All tested concentrations of Reklemel on cotton had similar growth parameters (plant height, shoot fresh weight and root fresh weight); however, the nematode population density (number of nematode eggs per gram of root) of both *M. incognita* and *R. reniformis* were significantly reduced by the application of 5, 50, and 250 ppm of Reklemel compared to water control. The results demonstrated that Reklemel at 250 ppm affected nematode egg hatch and concentration 5 ppm or higher significantly reduced nematode population density without altering plant growth parameters under greenhouse condition.

Introduction

Cotton (*Gossypium hirsutam*) is one of the most economically important crops in the United States. It is largely grown in the southern states stretching from Virginia in the east to California in the west. The U.S. lies behind China and India in cotton production but is the largest exporter of cotton in the world. It is estimated that cotton-based agro-industry contributes over $75 billion per year of economic activities in the U.S. generating thousands of employment opportunities ("World of Cotton," n.d.).

Cotton agro-industry is a multi-billion dollar per year industry; however, the profits from this agro-industry solely depends on the amount and the quality of the cotton yield at the farms. Besides being an economically attractive crop, cotton is attractive to many diseases and pests as well. If not managed properly, these diseases and pests can cause significant yield loss. In 2019, the Cotton Belt lost an estimated 6.7% of total cotton production due to various diseases. Plant-parasitic nematodes were the top most yield decreasing pests in the same year with an estimated 3.9% yield loss. *Meloidogyne incognita* and *Rotylenchulus reniformis* were the two most destructive plant-parasitic nematodes. The cotton yield loss was estimated to be 2.5% and 1.0% due to *Meloidogyne* spp. to *R. reniformis* respectively in 2019 (Lawrence et al., 2020).

Currently there are various strategies available for nematode management. The major nematode management practices include planting tolerant varieties, rotation with non-host crop and chemical controls. Managing nematodes with chemical treatments is the most effective control; however, public safety and environmental issues have restricted its wide applications, elevating the search for alternative eco-friendly nematicides. Salibro is a relatively new nematicide product from Corteva Agriscience™, the Agriculture Division of DowDupont. It is a sulfonamide compound having Reklemel as an active ingredient (formerly known as fluozaindolizine) (Thoden and Wiles, 2019). We evaluated Reklemel for *M. incognita* and *R. reniformis* management *in vitro* and *in planta*. Our results demonstrated that Reklemel could be a promising product for the management of these nematodes.

Materials and Methods

Nematode Culture

Nematodes (*M. incognita* and *R. reniformis*) eggs were extracted with 0.625% NaOCl followed by sucrose flotation and centrifugation (Jenkins, 1964). The collected eggs were either used for *in vitro* egg hatch assay, green house assay or incubated in sterile water for 3–5 days at 30˚C in a modified Baermann funnel to obtain second-stage juveniles (J2s). The hatched J2s were used for *in vitro* mortality assay following the methodology of Xiang et al. (2017).

Treatments

In the tests, four different concentrations of Reklemel i.e. 1 ppm, 5 ppm, 50 ppm, and 250 ppm were evaluated *in vitro* and *in planta* against *M. incognita* and *R. reniformis*. Sterile water was applied as non-treated control and fluopyram (5 ppm) as chemical control.

Egg Hatch Assay

The *in vitro* egg hatch assay was performed following the methodology of Nasr (2015) in 12 well polystyrene plates. The assay was a RCBD with four replicates and repeated once. A 100 µL suspension containing 200-250 *M. incognita* or *R. reniformis* eggs was added to each well and diluted with aqueous stock solution of Reklemel resulting in a final volume of 2 mL for each concentration. The plates were sealed with Parafilm and incubated in the dark at 25°C. The hatched J2s were counted at 1, 5, 7, and 9 days.

Juvenile Mortality Assay

The assay was performed similar to egg hatch assay with 150-200 active J2s/100 µL suspension. J2s were counted at 1, 3, 5, and 7 days, and data were collected for the number of normal, affected, and dead J2s based on the level of motility and body posture following the methodology of Thoden and Wiles (2019). After counting, the J2s were rinsed with sterile water and incubated in fresh water to determine the recovery as described by Faske and Hurd (2015). After one day of incubation, the J2 evaluation was made again.

Greenhouse Experiment

The greenhouse experiment was performed in 150 cm^3 plastic containers. Experiments were arranged in a RCBD with eight replicates and the entire experiment was repeated once. Two cotton seeds were planted in each container. One mL of *M. incognita* or *R. reniformis* egg suspension (2500 eggs/mL) and 1 mL of aqueous Reklemel solution with respective concentration were added to each pot at planting. Seedlings were thinned to one per container after emergence. Data were collected 35 days after planting (DAP). Plants were uprooted, plant growth parameters (height, shoot, and root fresh weight) and nematode eggs per gram of root were measured as described by Xiang et al. (2017).

Statistical Analysis

Data from egg hatch, J2 mortality assay, and greenhouse experiments were analyzed in SAS 9.4 using the PROC GLIMMIX procedure. The nematode population density (eggs/gram root) data required log-normal distribution transformation to satisfy the normal assumptions. LS-means were compared by Tukey- Kramer method at the significance level $P \leq 0.05$.

Results and Discussion

Egg Hatch Assay

All the concentrations of Reklemel tested had no significant inhibitory effect on *M. incognita* egg hatch; in contrast, Reklemel 1, 5, and 50 ppm stimulated *M. incognita* egg hatch more than the control over 9 days (Fig. 1A). However, *R. reniformis* egg hatch was significantly lowered by 250 ppm of Reklemel (Fig. 1B).

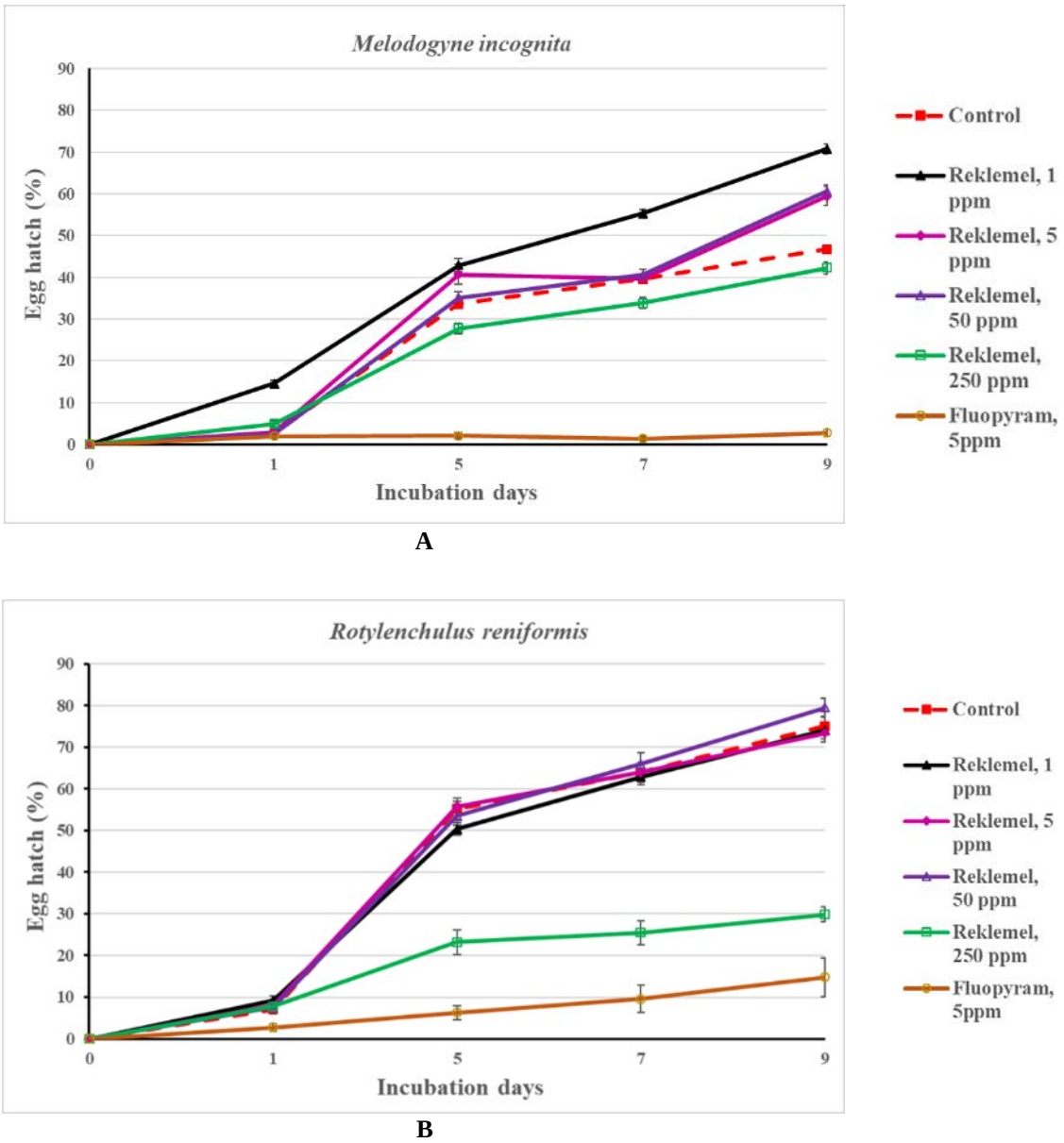
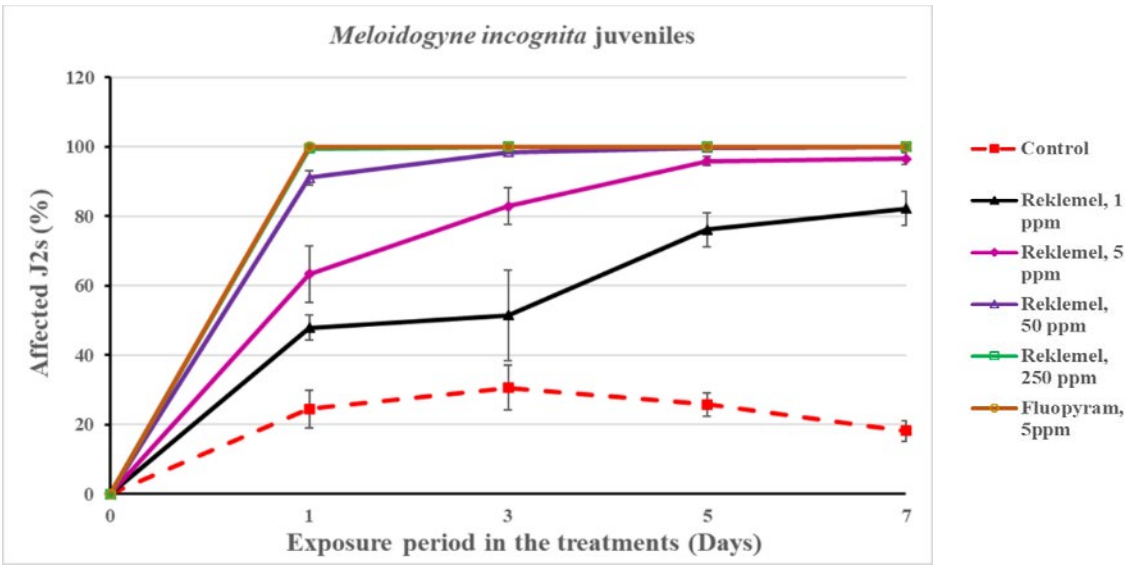


Figure 1: Percentage of *M. incognita* (A) and *R. reniformis* (B) egg hatch over 9 days with various concentrations of Reklemel. Data represents the LS-means of eight replicates (n=8) each containing 200-250 eggs per well.

Juvenile Mortality Assay

All concentrations of Reklemel were toxic to *M. incognita* J2s. The effects included abnormal movement (coiling, twitching, and deviation from normal sinusoidal movements) and formation of characteristic body postures. Healthy J2s showed active sinusoidal movements whereas affected J2s posed sluggish, coiling or twitching movement with 'L' or 'J' shaped body. The dead J2s were straight rods without any movement. The death of J2s were significantly higher in Reklemel, 250 ppm (at 3 days), 50 ppm (at 5 days), and 5 ppm (at 7 days) compared to control (Data not presented.). Figure 2A represents the combined data of affected and dead J2s at each time point. Juveniles were significantly affected by all the concentrations within 24 hours. After rinsing off the chemical, the J2s did not recover and continued to die (Fig. 2B).



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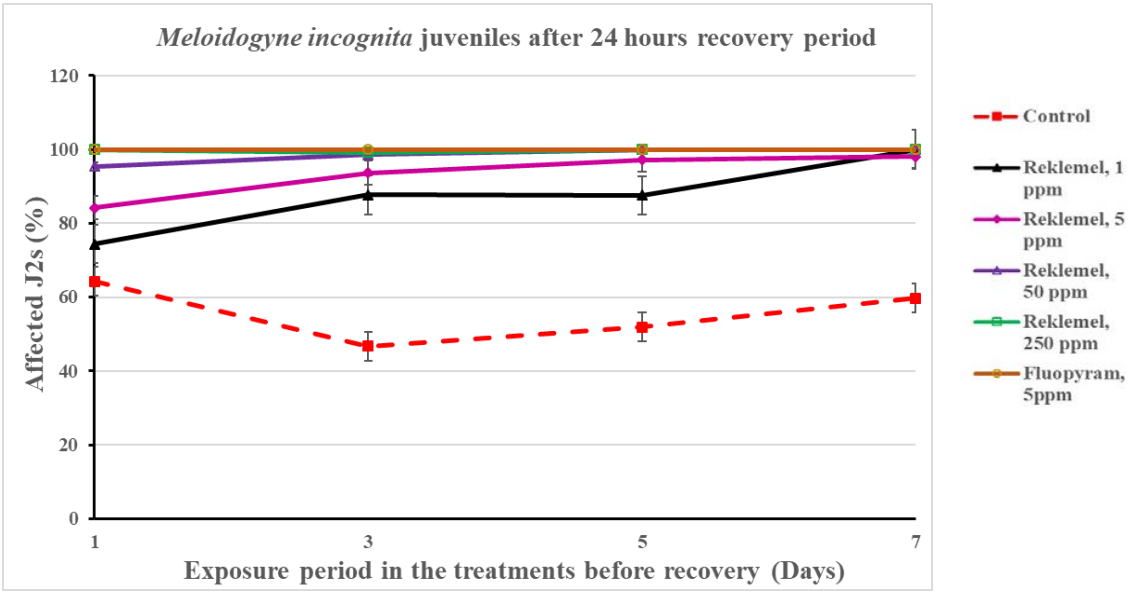


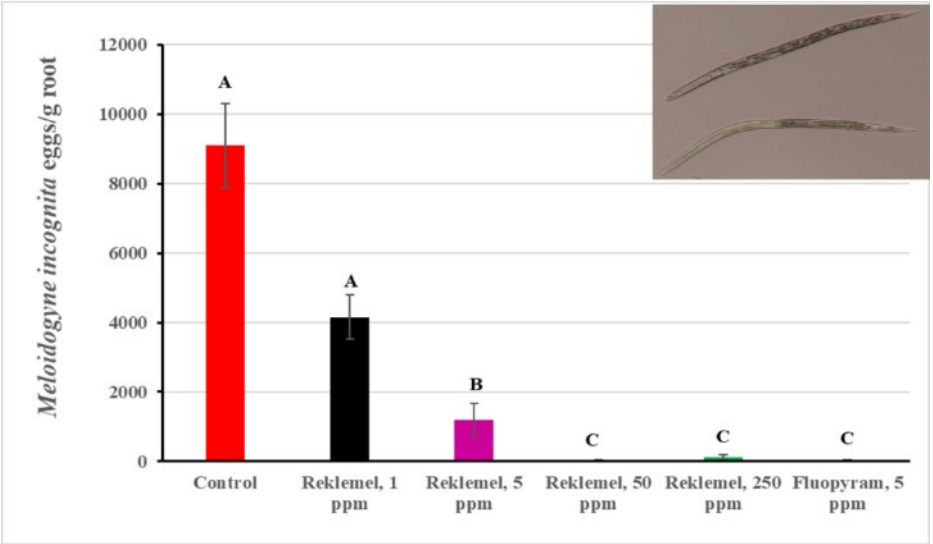
B

Figure 2: Percentage of J2s (both affected and dead) of *M. incognita* over 7 days (A), and the percentage of affected J2s after a day of recovery in water from different exposure times (B) in different concentrations of Reklemel. Data represents the LS-means of eight replicates (n=8) each containing 150-200 J2s per well.

The *in vitro* assay for *R. reniformis* J2 mortality was not performed because the J2s of *R. reniformis* were not actively moving as *M. incognita* J2s and tend to remain in characteristic 'C' shape irrespective of the medium. This nature of the *R. reniformis* J2s made it difficult to determine live, affected or dead J2s without stimulating with a probe.

Greenhouse Experiment

In the greenhouse experiments with *M. incognita* and *R. reniformis*, cotton plant growth parameters (height, root and shoot fresh weight) were not affected by any concentration of Reklemel measured at 35 DAP (Data not presented.). However, the nematode population density (eggs/gram of root) was significantly reduced by Reklemel 5, 50, and 250 ppm as shown in Fig. 3A (*M. incognita*) and Fig. 3B (*R. reniformis*).



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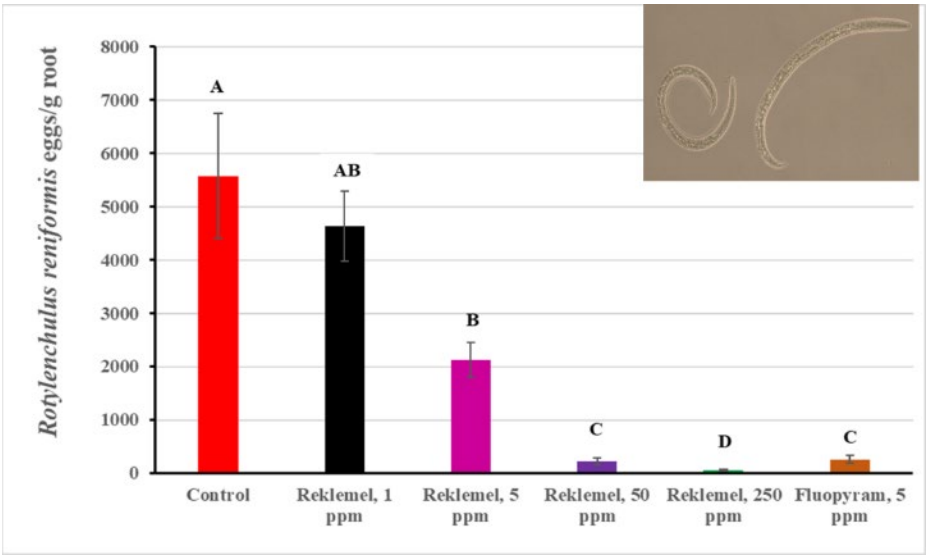

B

Figure 3: *Meloidogyne incognita* (A) and *R. reniformis* (B) population density (eggs/gram of root) in cotton at 35 DAP. Data represents the LS-means of 16 replicates (n=16) and the means with same letter are not significantly different at $P \leq 0.05$.

Summary

In the *in vitro* egg hatch assay, *R. reniformis* egg hatch was significantly reduced by 250 ppm of Reklemel; however, *M. incognita* egg hatch was not inhibited by any concentration of the Reklemel. Instead, the egg hatch of *M. incognita* was stimulated by 1, 5, and 50 ppm of Reklemel compared to the water control. Nonetheless, all of these concentrations of Reklemel induced toxicological effectes to *M. incognita* J2s within 24 hours of exposure. The effects were rapid with higer concentrations of Reklemel and irreversible as the affected J2s did not regain normal physiological activity in 24 hours of recovery period in normal water. In the greenhouse experiment, Reklemel at 5 ppm or higher significantly reduced the nematode population density (nematode eggs/gram root) compared to water control. The results indicate Reklemel could be a promising alternate to currently available nematicides.

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