Ability of systemic acquired resistance-saponin and a bacterial metabolite to reduce the soybean cyst nematode (*Heterodera glycines*) and the incidence of the sudden death syndrome (*Fusarium virguliforme*)

Weasam Adnan Aljaafri[1] Eman Rathi Husain[2] Sadeq Mohammed Ali [1]
Lecturer Lecturer Lecturer
[1]Department Plant of Protection, Faculty of Agriculture, University of Kufa, Iraq
[2] Technical Insitute, Al-furat-al-awast Technical university, Najaf-Iraq
E-mail: weasamaljaafri@yahoo.com

Abstract:

Experiments were conducted to to examine the ability of biological seed treatments reduce the Soybean Cyst Nematode and the incidence of Sudden Death Syndrome. Biological seed treatments included systemic acquired resistance (SAR)-Saponin, Bacterial metabolite, *Bacillus firmus, Burkholderia renojensis, B. subtilis,* and untreated seeds as control treatments. These seed treatments included four treatments included seed with no treatment (untreated seed), *H. glycines* alone*, Fusarium virguliforme* alone*, and H. glycines + F. virguliforme.* Tests included the standards Abamectin and Fluopyram. Treatments were arranged in a randomized complete block design with five replications. Parameter measured included effects on plant growth, nematode life stage development and the incidence of Sudden Death Syndrome. At 60 days, the biological seed treatments produced no significant negative plant growth effects. Treatments that included bacterial metabolite and SAR-Saponin significantly reduced *H. glycines*, cyst, juveniles, and eggs compared to the control. SAR-Saponin, and bacterial metabolite were statistically similar to the standards abamectin and fluopyram. Foliar disease was more severe in the treatments that included *H. glycines* compared with *F. virguliforme* than *F. virguliforme* alone. Foliar disease index reduced in treatments with saponin product compared to control in the bacterial metabolite and SAR-Saponin treatments respectively. Saponin and bacterial metabolite have shown potential for Sudden Death Syndrome and soybean cyst nematode management.

Key words: *H. glycines, F. virguliforme,* (SAR)-Saponin, Bacterialmet bolite, Sudden Death Syndrome (SDS).

Introduction:

Sudden Death Syndrome (SDS) is distinguished disease on the soybeans and devastating. In 1971 was the first appaeared in the United States at Arkansas, by H.J Walters who seen some plants with symptoms including chlorotic lesions in the field (27) Hirrel gave the name for this disease This disease was the worst in 2010, the loss evaluated around 4.7 million metric tons in the yield in the United States (6) Due to management options to SDS in the United States and need more helping to limit these losses of disease. The fungus that causes this disease is *Fusarium virguliforme* is causal agent that established in the USA (3) Recently, McLean and Lawrence in1993, also established that *F. virguliforme* could colonize with soybean cyst nematode their eggs. The SDS disease cycle starts with the infection stage of the roots for soybean by germinating chlamydospores, that are be the overwintering structure for

the fungus and can continue in high range of temperatures and soil cases. Later in the spring season, the chlamydospores can grow the mycelium of the fungus, that can be infected the roots of plants (21). As is seen from different researches that plants be infective with the same time of planting develop the worst foliar symptoms, but the older plants will be less susceptible to disease infection (10). After infection, symptoms develop as discolored of roots, blue spore masses can sometimes be showed on the taproot. (17, 27). Foliar symptoms consist of interveinal chlorotic lesions, which may eventually become necrotic. In addition, this disease has been shown associated with the soybean cyst nematode (*Heterodera glycines*). (16). Chlamydospores produce mycelium, which infect the plant roots. Plants infected at the time of planting develop the worst foliar symptoms, while older plants are less susceptible to infection. After infection, symptoms develop as discoloration of the roots and blue spore masses can sometimes be seen on the taproot. Foliar symptoms consist of interveinal chlorotic lesions, which may eventually become necrotic. Sudden Death Syndrome not only causes significant damage by itself, it also interacts with the soybean cyst nematode (*H. glycines*). The presence of *Heterodera glycines* in a field will lead to a greater severity of SDS. (21, 31, 32, 16). Saponins are secondary plant metabolites that occur in a wide range of plant species (12). They are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in response to pathogen attack. These compounds can also be regarded as ÒpreformedÓ, since the plant enzymes that activate them are already present in healthy plant tissues (23). The natural role of saponins in plants is thought to be protection against attack by pathogens and pets (24, 22). Saponins that have been studied in detail in relation to their potential role in plant defense against attack by phytopathogenic fungi are triterpenoid avenacins in oat roots and the steroidal glycoalkaloids a-tomatine in the leaves of tomato. Secondary metabolites involved in plant resistance against some of pathogens, some of higher plants are regularly exposed to microorganisms, at the level of both hypogeous organs and epigeous. Therefore, only a few of pathogens can cause diseases, because of the efficient plant innate immune system. Thus, as animals, compatibility of micro-organisms with a susceptible plant represents an exception in nature, while incompatibility with a resistant host is the rule (1). The latter condition depends on the plant defense machinery, a complex array of both physical inducible defenses and chemical preformed (13). Biological control is one of methods used to soil-borne pathogens by introduced microorganisms has been calculated for through 65 years (5), but over extreme of that time it has not been considered commercially practical. Biocontrol of nematodes was top studied (8). The expansion of biological dominance agents is also considered an active stand by nematode control on the vegetables (30, 15, 28). In biological control of plant-parasitic nematodes, the goal of many public- and private-sector research efforts has been to identify organisms that can be applied to the seed, planting furrow, or transplant medium to suppress nematode populations. The expectation is that a specific organism will act rapidly to reduce nematode populations and/or protect the growing seedling from damage. Persistence and proliferation of the organism in the root zone

has been considered a useful trait, but mainly to increase the level of nematode suppression in the crop to which the organism is applied (29). This strategy is referred to as inundation biological control (9); although it can be effective, it is not the only strategy for achieving biological control. Conservation biological control is the modification of the environment or existing practices to protect and enhance specific natural enemies or other organisms to reduce the effect of pests (9). Currently, numerous compounds are being examined for efficacy for management of the *Heterodera glycines* including Headsup, Thiabendazole, ILeVo). The purpose of study presented to using the potential saponin and bacterial metabolite as seed products applied as seed treatments as a possible biocontrol product for Sudden Death Syndrome and soybean cyst nematode management.

Materials and Methods:

Isolation and Identification of *F. virguliforme*

The isolate of *F. virguliforme* that used in this study was isolated from SDS symptomatic soybean roots from greenhouse at Mississippi State university, USA. The roots were washed in running tap water for three minutes then cut into 3-5 mm pieces with cortical and vascular tissues separated and placed on potato-dextrose agar (PDA) then identified according the morphological characteristics of *Fvirguliforme*. (20, 21, 2).

Examination of the relationship between *F. virguliforme* and Soybean Cyst nematode (*H. glycines).*

Cysts that had grown in a greenhouse at Mississippi state University were dislodging from the roots with a strong water spray and collect on nested sieves with pore sizes of 20- um and 100- um. Then eggs were released from the cysts using a modified seinhorst cyst crusher for 30 seconds. *H. glycines* second stage juveniles were extracted from soil using gravity screening and centrifugal flotation. (2, 18). Treatments were included control (untreated seeds), systemic acquired resistant product (saponin at 0.1 floz/cwt), systemic acquired resistant product (Saponin at 0.2 floz/cwt), abamectin as standard to compare our results with this product because already in the market as bio-nematicides, and bacterial metabolite at 3 floz/cwt that produce from specific bacteria. Seeds were treated with the appropriate experimental biological compounds and rates by Albaugh LLC (Table, 1) All biological seed treatments were used in a study that included *F. virguliforme* + *H. glycines, H. glycines* alone, *F. virguliforme* alone and an untreated control. All tests are planted into 15 cm dia. clay pots filled with an autoclaved soil included freestone fine sandy loam sand: (1:1, v/v). Plants are planted first by sowing one seed directly for each pot with 500 grams of the sterilized soil-sand mixture under greenhouse conditions and infected with 2000 eggs of soybean cyst and 0.75 gram of *F. virguliforme* produced on corn culture. At 60 days plants harvested then had been taken plant development and nematode development. In addition, the experiment included take root scan by using winRHIZO Pro software for root image acquisition and analysis included number of tips, number of forks, and number of crossings (2) The data for plant and nematode

population were analyzed using SAS with using a factorial arrangement of treatments in Random complete block design with 5 replications for each treatment.

Table1: biological seed treatments used in the experiments with soybean seeds.

Treatments	Product
1	Control- Untreated seeds
2	SAR1 - Saponin at 0.25 floz/cwt+ *Bacillus firmus* 1.25 floz/cwt
3	Bacterial Metabolite at 3 + Saponin at 0.50 floz/cwt
4	Abamectin at 3 + *Burkholderia renojensis* 2.0+ *B. subtilis* 1.5 floz/cwt
5	Fluopyram at 2.3) + Saponin 0.25 + Bacterial Metabolite at 3 floz/cwt

Measurements:

Growth of plants and development:

The total of weight for plants shoot plant roots, and plant height was measured after 60 days of planting.

Nematodes development:

From all these experiments with different treatments of seeds treatments counted, number of cyst, juvenile, and eggs for soybean cyst nematode and reproductive factor that had been measures by taken number of cyst + number of eggs+ number of juveniles divided by number of eggs that had been used in the test for each treatment. Foliar SDS disease severity was rated at 60 days using a 0-7 scale where 0-no symptoms, 1-mosaic mottling, 2-chlorotic mottling, 3-interveinal chlorosis, 4-interveinal chlorosis with leaf edge necrosis, 5-interveinal necrosis, 6- defoliation with leaflets separating from the petiole leaving the petiole attached to plant, 7- plant death (20, 2). Nematode population development was measured by the number of juveniles/ 500cm3 soil, number of cyst, and number of eggs at 60 days after planting.

Results*:*

F. virguliforme isolation

F. virguliforme used in this experiment was isolated from soybean plant that exhibited the symptoms for the sudden death syndrome to soybean roots in the greenhouse at Mississippi State university, USA. The identified and classification of *F. virguliforme was* identified after isolated from the roots. According to the methods that used by (20, 21, 2) during grew the *F. virguliforme* on potato dextrose agar with blue-pigmented growth on PDA (figure 1), then identified according the morphological characteristics of the organism from the vegetative structures included microconidia,macroconidia,chlamydospores, and conidiophores of *F. virguliforme* (Figure1)

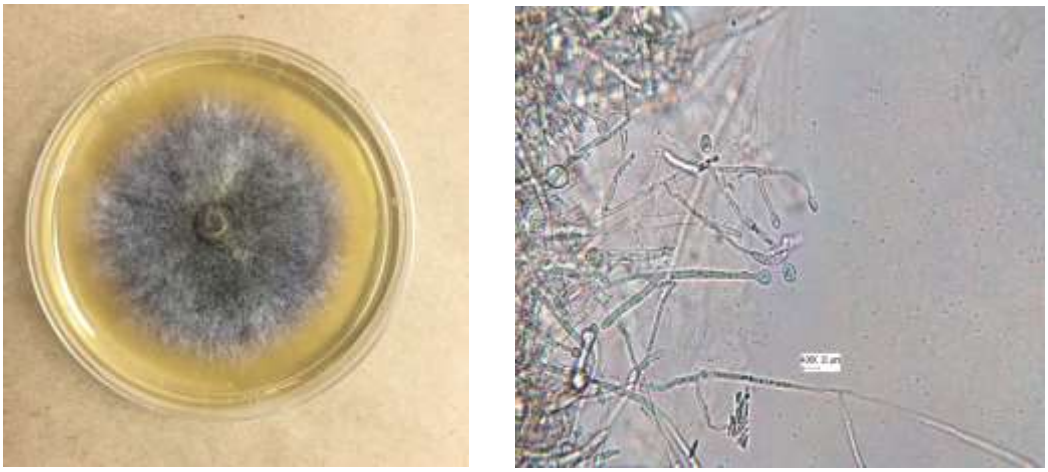

Figure1: Culture of *F. virguliforme* isolated from the soybean roots. Characteristics growth structures of *F. Virguliforme* identified under microscope *a4q*included Conidiophore, Chlamedospores, Microconidia and Macroconidia of *F. virguliforme.*

Effect of Saponin-SAR product and a bacterial metabolite seed treatment that infested with *F. virguliforme* and *H. glycines* on plant growth and development.

There was no negative effect on plants growth and development happened by *H. glycines* with *F. virguliforme* when using the biological seed treatments compared with control without treatment (Figure2). Results referred to mention a positive effect for improving plant growth with any treatments from biological seed treatments include saponin, *Bacillus firmus,* bacterial metabolite, abamectin, *Burkholderia renojensis, B. subtilis,* and Fluopyram. There was a significant effect on number of pods and weight of pods between the untreated control, *H. glycines* alone, *F. virguliforme* alone, and *F. virguliforme* + *H. glycines* combination. Most of the biological seed treatments in the *F. virguliforme* + *H. glycines* combination no produced effects compared to the control. Most of the treatments were significant to increased plant growth included plant weight, height, and root weights improved plant weight from 12.9 grams in control treatment to 22 grams in SAR1 - Saponin at 0.25 floz/cwt+ *Bacillus firmus* 1.25 floz/cwt. Also, the same treatment gave higher of height of plants and root weights compared to control treatment. In addition, other treatments were giving significant different results to improve plants growth compared with control treatment. Most of the treatments gave the similar results to fluopyram and abamectin that used as standard to be compared our results with these products.

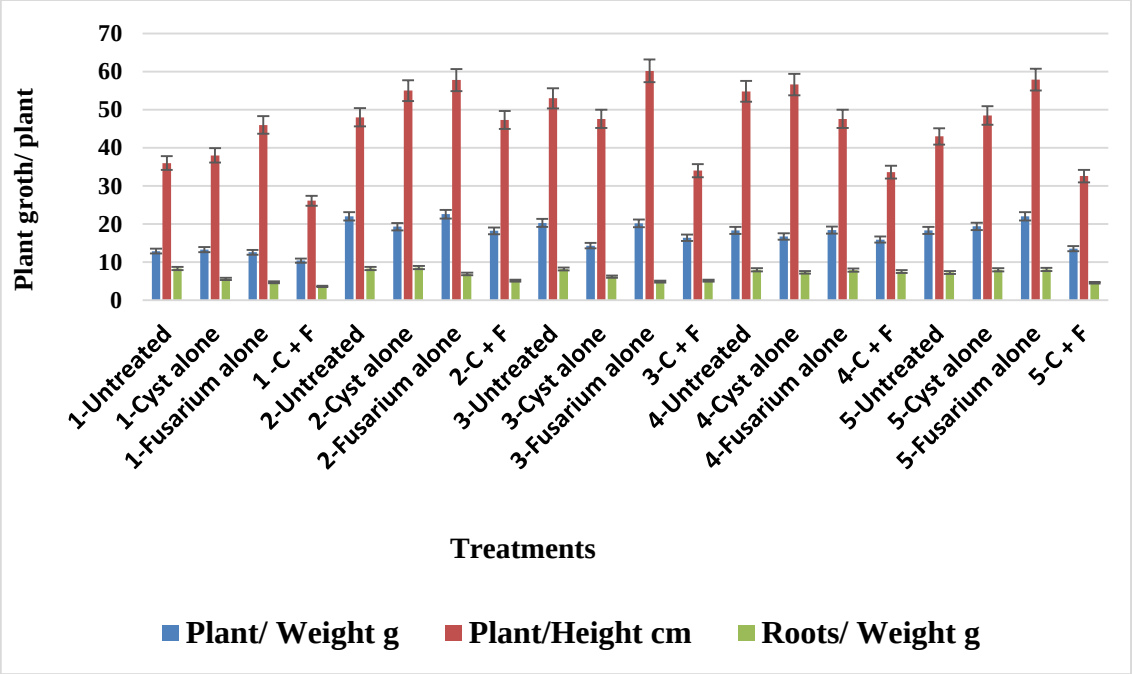

Figure 2: Effect of saponin SAR-product and bacterial metabolite as seed treatments on soybean plants growth (plant weight, plant height, and roots weight) infested with *H. glycines and F. virguliforme.* Data are means of the 5 replicates for each treatment 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.

P-Value, Plant/ Weight grams, 0.0001, LSD $_{0.05}$ = 2.0342

P-Value, Plant/ Height.cm^{-3}, 0.0021, LSD $_{0.05}$ = 2.768

P-Value, Roots weight.g^{-1}, 0.032, LSD $_{0.05}$ = 1.0653

Effect of Saponin-SAR product and a bacterial metabolite seed tratment that infested with F. virguliforme and H. glycines on nematode life stages development.

At 60 days, the numbers of *H. glycines life stages* were reduced by most of the biological seed treatments compared to the untreated control. As have been seen in this experiment, most of the treatments that included *H. glycines and F. virguliforme* reduced number of cysts. In the treatment Fluopyram at 2.3) + Saponin 0.25 + Bacterial Metabolite at 3 floz/cwt reduced number of cysts from 546.23 in the control treatment to 76.4 cyst/plant in the treatments that have *H. glycines and F. virguliforme.* In contrast, in the same treatment with *H. glycines* alone, the number of cysts was 876 cysts/ plant in control treatment reduced to 298.23 cyst / plant in treatment Fluopyram at 2.3)+ Saponin 0.25 + Bacterial Metabolite at 3 floz/cwt with *H. glycines* alone. Other treatments also were significant to reduced number of cysts compared to untreated treatment with different significance effect depended on different treatments (Figure 3).

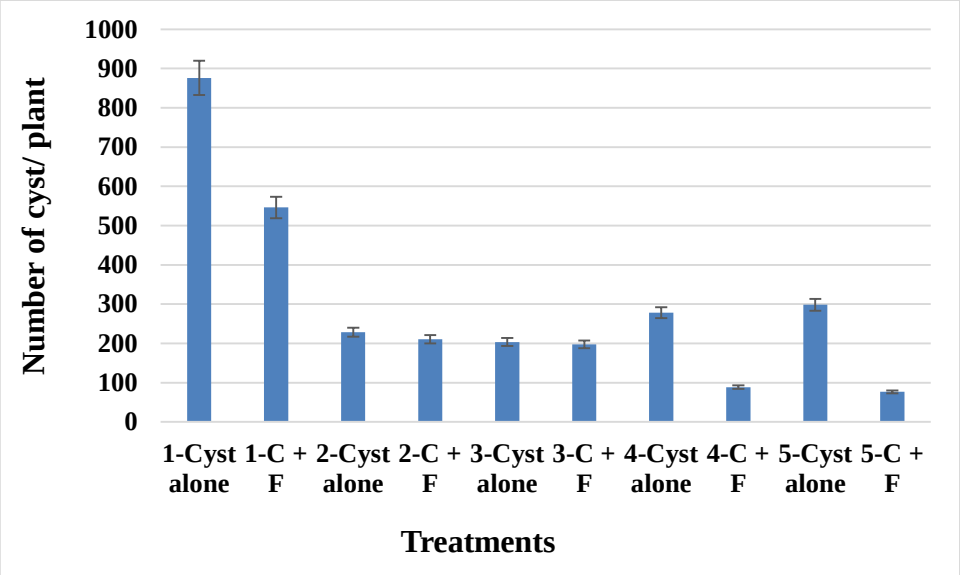

Figure 3:Effect of Saponin SAR-product with bacterial metabolite as seed treatment on *H. glycines* number of cysts development on soybean infected with *H. glycines and F. virguliforme.* Data are means of the 5 replicates for each treatment 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.

P-Value, Cysts/ plant, 0.0001, LSD $_{0.05}$= 68.057

In addition, the numbers eggs and juveniles of *H. glycines* were also reduced by most of the treatments (Saponin, *Bacillus firmus*, bacterial Metabolite, abamectin, *Burkholderia renojensis*, *B. subtilis*, and Fluopyram.) compared to the untreated control. In the treatment, SAR1 - Saponin at 0.25 floz/cwt+ *Bacillus firmus* 1.25 floz/cwt was reduced number of eggs from 16342.34 in untreated treatment to 2873.34 eggs/ plant. Other treatments were significant also to reduce number of eggs compared to control treatment (Figure 4).

Also, there was a significant difference to reduce the number of juveniles in the treatment (Fluopyram at 2.3) + Saponin 0.25 + Bacterial Metabolite at 3 floz/cwt) with treatment *F. virguliforme* + *H. glycines* combination from 12657.3 juveniles per 500cm3 soil in the control treatment to 188.32 juveniles / 500 cm3 soil. In addition, other treatments also had significant difference to reduce number of juveniles in both treatment with *H. glycines* alone and *F. virguliforme* + *H. glycines* combination compared to control treatment (Figure 4).

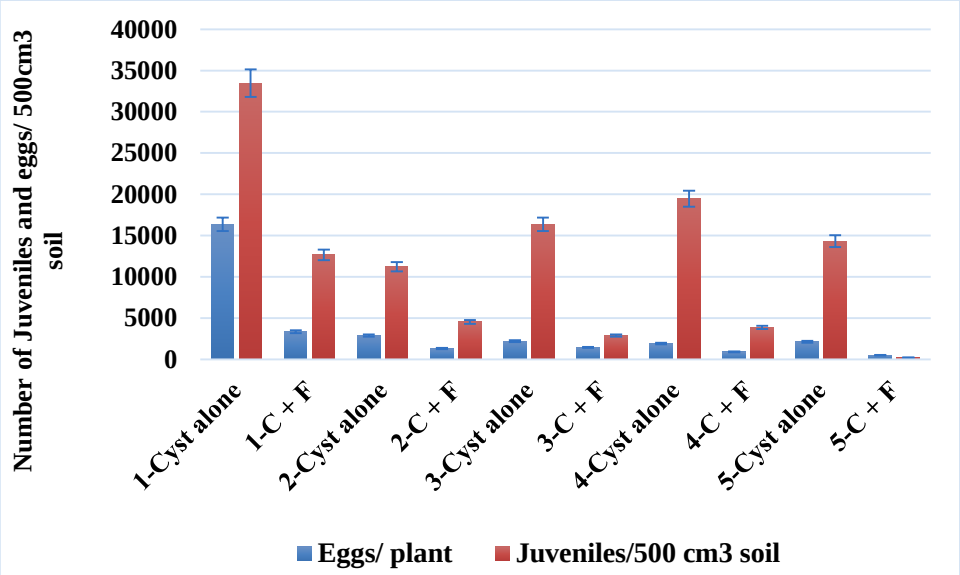

Figure 4 :Effect of Saponin SAR-product with bacterial metabolite as seed

treatment on H. glycines life stage development (number of eggs and juveniles per 500 cm3 soil) on soybean infected with H. glycines and F. virguliforme. Data are means of the 5 replicates for each treatment 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05. P-Value, Eggs/ plant, 0.0001, LSD 0.05= 325.67, P-Value, Juveniles/ 500 cm3 soil, 0.0021, LSD 0.05= 2310.49

Reproductive factors for different treatments also had been shown significant difference to reduce the number of productive factors for nematode development with different significant value. In the treatment (Fluopyram at 2.3) + saponin 0.25 + bacterial metabolite at 3 floz/cwt) was 0.302724 compared to 6.61976 in the untreated seeds. In addition, other treatments had been seen different significance effect on reproductive factors with other treatments compared to control treatment (Figure 5).

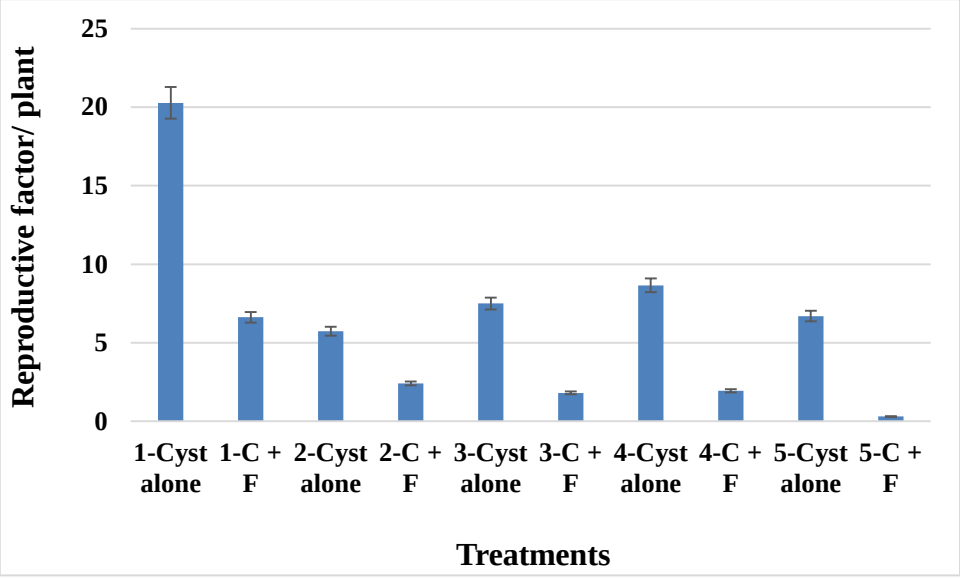

Figure 5: Effect of Saponin SAR-product with bacterial metabolite as seed

treatment on *H. glycines* life stage development (Reproductive factors) on soybean infected with *H. glycines and F. virguliforme.* Data are means of the 5 replicates

for each treatment 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05. Reproduction Factor (RF) = Eggs+ Cyst + Juveniles at 60 days/ 2500 eggs. P-Value, reproductive factor =
with *F.* Symptoms of the Sudden Death Syndrome developed only in pots that included *F. virguliforme alone* and *F. virguliforme + H. glycines* combinations. Foliar leaves symptom ratings at 60 days after planting were significantly more severe in the *F. virguliforme + H. glycines* combination treatments compared to *F. virguliforme* alone. Most of the treatments significant reduced foliar symptoms *virguliforme alone* compared to untreated seeds (Figure 6).

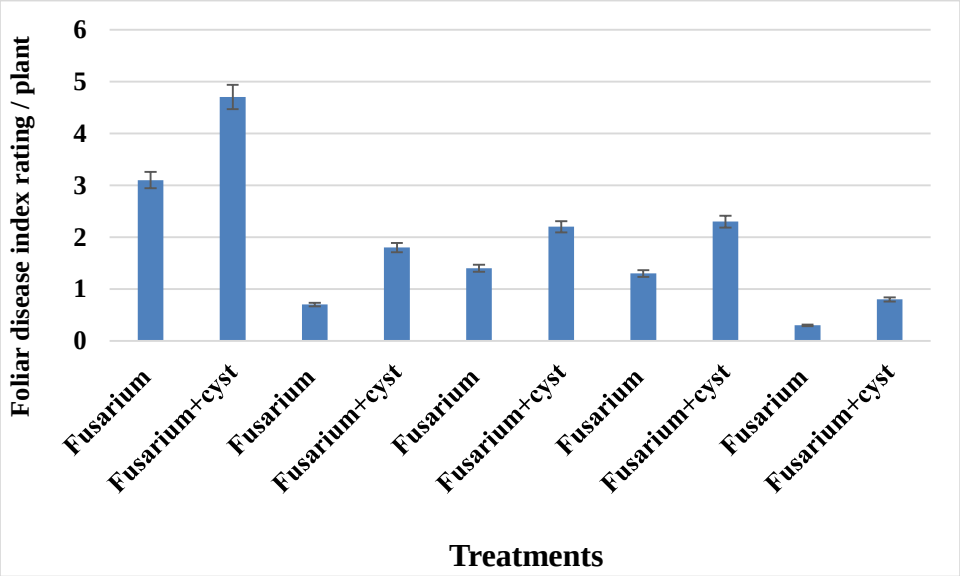

Figure : 6 Foliar disease index rating with biological seed treatments. Foliar SD.

dise severity was recorded at 60 days after harvest using a 0-7 scale, where 0-no symptoms, 1-mosaic mottling, 2-chlorotic mottling, 3-interveinal chlorosis, 4-interveinal chlorosis with leaf edge necrosis, 5-interveinal necrosis, 6- defoliation with leaflets separating from the petiole leaving the petiole attached to plant, 7- plant death. Data are means of the 5 replicates for each treatment after 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05. P-Value = 0.0013, L.S.D $_{0.05}$ = 1.02In results for root-scan by WinRHIZO optical scanner (Table 2) had been shown different results most of them were significant to improve root growth compared to untreated see As has been seen from the results for number of root tips, forks, and crossings have been shown significant different compered to control treatment. In this study, root tips, forks, and crossings densities differed significantly with biological seed treatments especially in the treatments (untreated, H. glycines alone, F. virguliforme alone) compared to untreated seeds. However, there was numerically differences between treatment with H. glycines and F. virguliforme combinations and untreated, H. glycines alone, F. virguliforme alone) although were significant compared to untreated seeds. In this experiment have two associated with plants infected by two pathogens (H. glycines and F. virguliforme). (Table 2). pathogens inciting disease at the same area (root tip), may be another reason why less cysts.

Table 2: Effect of Saponin SAR-product with bacterial metabolite as seed treatment on root development by root-scan by WinRHIZO optical scanner infested with *H. glycines and F. virguliforme.*

Treatments	Tips	Forks	Crossings
1-Contril-Untreated	3349.8	3665	287.6
1-Control-cyst5	2154	2138.2	140.4
1-Control-Fusarium	1883.8	2202.4	224.4
1-Control-F+C	1627.8	1647.2	135.4
2-Untreated	4508.4	5384.4	410.8
2-Cyst	3293	4503	300.8
2-Fusarium	3432.6	3781	234.2
2-Fusarium+cyst	2316.2	3187.6	182.6
3-Untreated	2925.4	4378.6	1707.6
3-Cyst	2239.4	3157.2	263.2
3-Fusarium	7825.6	16537.6	1499.4
3-Fusarium+cyst	2130	11632	369
4-Untreated	8414.4	14297.4	1150
4-Cyst	6440.4	14730.8	766.8
4-Fusarium	8067.6	14921	1200.8
4-Fusarium+cyst	6812.2	7356.6	869
5-Untreated	8022.2	18918.2	1719.6
5-Cyst	7042	17288.6	1579.6
5-Fusarium	7784	16404.4	1342.6
5-Fusarium+cyst	5485.6	9963.6	944.6
P-value	0.0001	0.0034	0.0012
LSD $_{0.05}$	**97.34**	**123.47**	**39.12**

Data are means of the 5 replicates for each treatment 60 days. Means compared by using Fisher`s protected least significant difference test at 0.05.

Discussion:

F. virguliforme isolated from the soybean roots that were showing symptoms of Sudden Death Syndrome. *F. virguliforme* produced characteristic of mycelium with blue-pigmented, grayish white or bluish color on PDA medium, these characteristics of mycelium had been seen different study for *F. virguliforme* as identified for fungus by (20, 21, 2). None of the biological candidates had a negative impacted plant development when challenged by *H. glycines,* and Sudden Death Syndrome(SDS). Most of treatments with Saponin product and bacterial metabolite had been shown significant difference to improve plant growth compered to control treatment with the *H. glycines* alone compared with the *F. virguliforme + H. glycines* combination. None of the Saponin and bacterial metabolite seed treatment products screened had no impact on plant development including soybean weight of plant, height of plant, and weight of roots in soils infested with *H. glycines and F. virguliforme*, untreated control, *H. glycines* alone, *F. virguliforme* alone, and *F. virguliforme + H. glycines* combination when compared with untreated seeds. This result was agreed with Aljaafri (2) had been shown activity for different seed treatment to improve plant growth for soybean with different biological seed treatments including some of bacte-

ria like *Bacillus spp*. and other products. In addition, the experiment had been shown activity to reduce number of life stages for nematode. This reduction in the life stages of *H. glycines* when both *F. virguliforme* and *H. glycines* were present on soybeans has been recorded in the literature 19, 2). In most of treatment have seen low number of cyst and juveniles in most of treatment especially with *F. virguliforme* and *H. glycines* together. These results agree with *F. virguliforme* has been shown to parasites on soybean cyst nematode and prevent nematode from produce syncytium on the feeding site of soybean roots. (19, 2). The saponins produced by oats and tomato have been studied in detail in relation to their potential role in the defense of plants against phytopathogenic fungi (23).

As is seen biological seed treatments that in the marketed for management of H. glycines are Avicta® (abamectin, Syngenta), Clariva® (Pasteuria nishizawae, Syngenta), and VOTiVO® (Bacillus firmus, Bayer CropScience) have activity to reduce number of life stages for soybean cyst nematode. These results agree with Aljaafri (2) to prove ability of these product to reduce number of life stages for H. glycines. In addition, the possibility of Burkholderia sp as biocontrol agents against various plant pathogens have been recorded (7, 14).

Foliar leaf symptoms were significantly more severe in pots that included F. virguliforme + H. glycines combination treatments compared to F. virguliforme alone. The symptoms of the SDS developed after 60 days after planting of soybean in the greenhouse condition. These results agree with many researchers found the same results to reduce the effect of disease severity with F. virguliforme and H. *glycines* combination (19, 2). Saponin and the bacterial metabolites was not significantly different from the fungicide/nematicide product fluopyram. Fluopyram is a fungicide that have been shown activity against Sudden Death Syndrome (4, 2). In addition, this product also had been shown activity to reduce H. glycines on soybean. Foliar leaf symptoms were significantly more severe in the pots that included both pathogens F. virguliforme + H. glycines combination treatments compared to F. virguliforme alone. Most of the treatments significantly reduced the foliar symptoms with F. virguliforme alone to untreated seeds with saponin product and bacterial metabolite were statistically different in the number between F. virguliforme alone and F. virguliforme + H. glycines combination for foliar disease symptoms severity, however, both treatments were significantly different compared to untreated seeds. Foliar symptoms were increased in the treatments that included F. virguliforme + H. glycines combination. These results were agreed with Aljaafri (2) had been shown activity for B. rinojensis as a biological agent against F. virguliforme and H. glycines. Some of Burkholderia sp. have been used to control seedling damping off disease on cotton incited by Rhizoctonia solani (33).

The WinRHIZO optical scanner is an efficient method that allow image analysis and examination of the root morphological traits. This method was used for screening of root traits of soybean grown under H. glycines and F. virguliforme infections. Plant roots optimize their root to acquire essential nutrients and water. Number of root tips, forks, and crossings have been shown significant roles on root structure be-

cause they have potential to encourage penetration through soil layers, that leads to good effects to get water and essential nutrients to plant. In this study, root tips, forks, and crossings densities differed significantly with biological seed treatments especially in the treatments (untreated, H. glycines alone, F. virguliforme alone) compared to control. (25, 26, 2). Also, the number of roots tips can be decreased with lower number of cyst of H. glycines alone infection and more number of roots tips with present of H. glycines that could be less severity of SDS (34, 2) The objective of this study was to identify a viable biological candidate that would be efficacious on soybean cyst nematode (H. glycines) and Sudden Death Syndrome (F. virguliforme) All the biological products performed statistically better than the control in regard to improve plant growth and reducing cysts, eggs, and juveniles, and reproductive factors with different treatment for biological seed treatments as well as the overall nematode reproduction. Also, differences in number of cysts, eggs, and juveniles in the treatments with H. glycines alone was higher numbers than F. virguliforme + H. glycines combination. Foliar leaves symptoms were significantly more severe in the pots that included both pathogens F. virguliforme + H. glycines combination compared to F. virguliforme alone treatment. Most of these biological controls have shown similar results to the standard nematicide seed treatment product. Future research should focus on using different modes of action (fungicides and nematicides) that would promote both sustainable and economical protection in reducing both SDS and H. glycines.

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