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Integrated Management of Cereal Cyst Nematode *Heterodera avenae* on Wheat Crop Under Centre Pivot Irrigation System in Tabuk, Saudi Arabia

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ABSTRACT

Cereal cyst nematode (CCN), *Heterodera avenae* was reported on the wheat crop grown under centre pivot irrigation system in Gassim region in the mid of Saudi Arabia; soon after it spread out to other areas in Al-Kharj and Hael. The estimation of wheat yield reduction by the CCN in Saudi Arabia ranged 40-92% on sandy soil. CCN was observed at TADCO wheat pivots on Feb 2006. A field survey was carried out in the same month shown the presence of severe infestations of this nematode in small batches of total 14 pivots. It was not clear how this nematode pest was introduced to the kingdom of Saudi Arabia: apparently, it was introduced with the imported potato seeds. Soil samples were collected from the damaged areas in each pivot to determine the level of infestations with CCN. Integrated Cereal Cyst Nematode Management Plan was implemented which demonstrated a great degree of success and minimized the expansion of infested areas with CCN and minimized yield reduction to around 4.1% on the mean total wheat production at TADCO for the period 2006-2011. CCN management plan included: monitoring CCN infestations on yearly basis, the application of systemic nematocide on the infested spots of each pivot. Also, the application of granular NPK 14-38-10 and K2SO4 fertilizers, and the implementation of crop rotation mainly alfalfa by direct drilling using No-Till method for wheat sowing, use certified wheat seeds and Machinery Sanitation.

Wheat germplasms resistant to CCN were investigated using 29 genotype seeds samples of the 3rd Elite Bread Wheat from the CIMMYT nurseries, apparently, entry # 15 is resistant to CCN.

INTRODUCTION

The Cereal cyst nematode (CCN), *Heterodera avenae* Wollenwebber has been reported to cause economic losses on wheat & barley crops worldwide in North America, Europe, and temperate areas in the Northern & Southern Hemispheres. It caused serious losses in the Middle East countries with cold winter such as Saudi Arabia, Syria & Turkey [1-4].

CCN was detected for the first time in the Kingdom of Saudi Arabia (KSA) in one farm in Gassim region causing damage on the wheat crop grown under centre pivot irrigation system [1]. Soon after few years, it was reported in Al-Kharj farms area near Riyadh & in Hael region [5,6]. Reports of that heavy infestation with CCN in sandy soil reduced wheat yield by 40-92% and 17-77% in barley [2].

Tabuk Agriculture Development Co. (TADCO) grows field crops such as spring wheat, alfalfa, corn, potato and onion into the centre pivot irrigation system, each pivot is usually 50 Ha. Wheat pivots of TADCO were free from CCN infestations until it was detected on one pivot on February 8, 2006, showing yellow batches of stunted wheat plants which, we identified in the Lab as cereal cyst nematode.

MATERIALS AND METHODS

To identify the cause of stunted yellow plants observed on the yellow patches into the wheat pivots and to determine the level of infestation, soil samples were collected by the research personnel from the damaged areas of each pivot, each sample is

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around one liter of soil with around 10 plants packed into a polyethylene bag, then a label with location & date was assigned to each sample and then the samples were sent to TADCO's lab for further inspection.

The nematode juveniles were extracted from the soil samples using the modified centrifugal-floatation technique [7] as follows: Put the soil sample in a bowl and clean it from stones, excluding the roots in a separate beaker for separate inspection. Take 250 ml of cleaned soil and put it in a plastic bucket (10 L.) and add one liter of water, soak it for 3-5 minutes then add 1.5 L water and stir it thoroughly to break the soil aggregates and wait for 30 seconds to allow soil particles to settle downwards. Pass the floated material with water over sieve #2 (500 µ) & sieve #3 (250 µ) arranged over each other inside a pan to exclude the soil & debris residues over the sieves and to collect the nematodes with the water in the ban. Pass the collected water in the ban over sieve #7 (45 µ) to catch the nematodes, and then gently transfer the residue with water to a small beaker. Stir the residues and the nematodes into the beaker & pour it into 4-6 centrifuge polyethene test tubes. Add water to equalize the volume 0.5 cm from the top and spin tubes at 3200 rpm for 4 minutes. Discard supernatant retain the residue, and then add an equal volume of sugar solution (45%) to the residue in each test tube 0.5 cm from the top. Stir thoroughly with a spatula to mix it with the sugar solution and shake well, then spin at 3200 rpm for 2 minutes. Pour the supernatant into a clean sieve #7 (45 µ), wash thoroughly to remove the sugar and transfer the nematodes to a graduated beaker (50 ml). Take one droplet of the nematode solution on a glass slide, cover it with a coverslip and examine under a microscope at 100X magnification which should be typical to CCN J2's [8]. Using a dropper stopper mix the sample into the beaker and get 1 ml of the nematode solution, then put it on a counting dish and counts the CCN J2's. Repeat counting on another 1 ml sample and take the average numbers per 1 ml sample. The number of 2nd stage juveniles per 1 ml is multiplied by 50 then by 4 to get the number of 2nd stage juveniles per one liter of soil.

We checked another air dried sub-soil sample of 100 gram for the presence of old brown lemon-shaped cysts using flask floatation method [9], and the numbers were expressed as the number of cysts/100 gram or number of eggs/gram of soil. Cysts were picked in a small dish then transferred and crushed over aluminum plate; the crushed cysts were transferred with aid of gentle water into a test tube with a total volume of 20 ml water and stirred with a mechanical rod mixer. Two (1) ml aliquots samples were removed by manual pipette to count the eggs and expressed as the number of eggs/cyst.

Roots were washed free from soil & stained with 0.5% hot acid lactophenol cotton blue and processed for extraction & counting number of J2's per root system or per gram root [9].

Since the wheat cultivar, Yecora Rojo is susceptible to CCN infestations, an integrated short term & long-term management plan was put forward to manage CCN infestations at TADCO to avoid the potentially high yield loss in coordination with the top management & production managers. The short-term management plan included, the survey of damaged pivots, sketch drawing of damaged areas, treatment of CCN damaged areas at each pivot with systemic granular nematocide Furdan 10G or Nemacur 10G at the rate of 2 kg/1000 square meter 35-40 days after sowing to stop further crop damage by the nematode. To raise the wheat crop tolerance to CCN infestations, top dressing of the whole planted pivots with granular NPK fertilizer 14-38-10 at the rate 150-200 kg/ha and granular Potassium Sulphate fertilizer 100 kg/ha after herbicides spraying program 45-60 days after sowing. Control other pests, diseases & weeds on all TADCO wheat pivots such as Aphids, Leaf Minor & grasses as per the recommended program through pesticides injections or herbicides spraying to the whole area of the damaged pivots.

In the long-term management plan, we conducted a yearly survey for the CCN infestations before sowing the wheat crop to determine the pivots infestations with dormant cysts and to check the suitability of each pivot for wheat sowing. Also, we surveyed the fields (3-5 weeks after sowing) to determine the degree of infestations expansion on old infested wheat pivots to take a proper control action. Soil cultivation was conducted after wheat harvest by two rounds of chisel plough to expose the fungus pathogens and nematodes to solar radiation during summer months before planting summer crops. Sanitation measures were carried out to prevent the dissemination of CCN through farm machinery such as combine harvesters, land preparation equipment etc. using air & water pressure for cleaning these machines after farm operations of infested pivots and before entry of new pivots. Crop rotation of two-years duration was applied to non-host crops for the CCN such as alfalfa or potato or onion to lower the density level of the nematodes in the soil to be safe for wheat production. During the rotation, attention was carried out to control grass weeds such as ryegrass & downy brome grass during the period of growing the non-host crops. We had directed our efforts to look for wheat cultivars resistant to CCN nematode which were acceptable by the end user.

To search for wheat cultivars resistant to CNN, we tested 29 wheat genotype seeds samples of the 3rd Elite Bread Wheat (3rd EBWYT) sent to TADCO from CIMMYT nurseries in 2008 through the ministry of Agriculture in Riyadh. We prepared the infested soil for the trial from naturally infested soil collected from random locations of the infested pivot A3P19. Mix NPK granular fertilizer 14-38-10 at the rate of 2.5 gram/liter of soil. Fill the infested soil in one-gallon black plastic bags; place them on a clean area outside A3P19 Pivot in preparation for sowing wheat seeds samples. Arrange the bags in the trial as R.B.D. design with three replicates/genotype and put the bags into holes in the soil surface to protect them from direct sun heat, and then sow the seeds (10/bag) on Jan 22, 2008. Irrigation and fertilizers application were carried out frequently to match field application of wheat pivots. We measured the population density on the soil mix before sowing the seeds by taking twelve random soil samples from the soil mix and analyze them in the lab; population density was 4.88 eggs/gram of soil **(Table 1)**.

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Table 1. Initial population density of cereal cyst nematode before starting bread wheat varietal trial for CCN in 2008 season.

Replicate No.	No. of Cysts/114 Gram of Soil	No. of Eggs/Cyst	Eggs/Gram of Soil
1	13	41	4.67
2	11	47	4.53
3	8	48	3.36
4	14	50	6.14
5	9	69	5.44
6	7	33	2.02
7	8	64	4.49
8	10	53	4.64
9	8	44	3.08
10	12	38	4
11	16	53	7.43
12	15	72	9.47
Mean	10.92	51.00	4.94

So, Number Eggs/gram of soil = (10.92 x 51)/114= 4.88.

RESULTS

Lab inspection on the soil samples taken from infested pivots shown the presence of CCN 2nd stage juveniles (J2) into the soil extract **(Figure 1a)**; the larvae is around 0.5 mm length with sharply pointed tail and well developed median bulb. The presence of Lemon Shaped brown color cysts with a length 0.5 - 1 mm **(Figure 1b)** with viable eggs inside confirm the infestation with CCN on the damaged wheat pivots [8]. We observed yellow batches of stunted wheat plants, with less growth & size than normal healthy and green plants **(Figure 2a)**; the Infested plants were with stunted & thickened root growth **(Figure 2b)** which was our guide tool for sampling & surveying infestations. We observed the damage was more severe in light sandy soil than loamy soil.

Analysis results on the soil & plant sample collected on Feb 8, 2006, from the CCN infestations at A3P19 pivot is presented in **Table 2** along with a comparison on the soil and plants sample sent from a sister company NADEC and analyzed on Jan 2001. The results showed very high numbers of the different stages of CCN on both samples, and the soil infestation level at NADEC was higher than at TADCO apparently due to the accumulation of infestation with this nematode on old infested pivot # 36. Both samples showed a high level of eggs/gram of soil & 2nd stage juveniles above the threshold level 5 eggs & larvae/gram soil. The wheat seedling roots were less than 0.2 gram, but it contained high numbers of juveniles per root system which caused yellowing & stunted growth of plants seen on the damaged patches of each pivot. Both samples showed a very low-level of eggs/cyst.

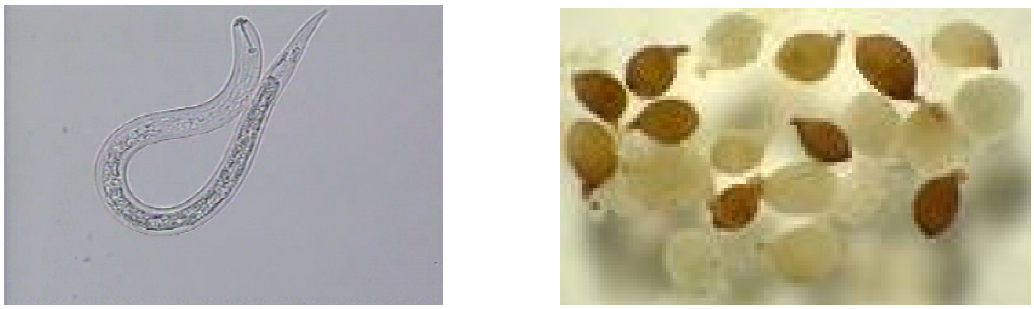

Figure 1. 1a) 2nd Stage juvenile of CCN (J2); 1b) Matured Cysts of CCN are Brown color with lemon shape.

Figure 2. 2a) Yellow patches of stunted Wheat plants at A3P19; 2b) Stunted wheat roots on the right and normal roots on

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the left.

Table 2. Summary table for the different CCN stages found on the new infested pivot at TADCO on Feb. 2006 with a comparison of high CCN infestation at one NADEC wheat pivot in Jan 2001.

Analysis	TADCO Feb 8, 2006 (A3P19)	NADEC Jan 12, 2001 (Pivot # 36)
Number of 2nd Stage juveniles (J_2) / Liter of Soil	5920	11849
Number of Cysts per 100 grams of Soil	13	70
Number of Eggs per Cyst	28	43
Number of eggs/gram of soil	3.64	30.1
Number of 2nd Stage juveniles (J_2) / gram Root	476	ND
Number of 2nd Stage juveniles (J_2) / one Root	31.7	108.3

Survey for the Infestations with CCN in 2006 cropping season showed the presence of CCN infestations on 14 pivots; the level of infestation at each pivot is shown in the **Table 3**. Very high numbers of 2nd stage juveniles were found in the soil samples collected from the soil around the roots of damaged plants. The level of infestation was very high (16800 – 39040 J2/L of soil) on the plants of five pivots, and high infestation into seven pivots (4480-16000 J2/L of soil; two pivots were with moderate infestations<4000 J2/L of soil. The infested pivots were: A07P16, D03P05, F03P05, B05P15, G03P06, J21P22, J41P81, J45P83, D04P08, A06P14, K02P06, A07P15, K06P15 & A03P19, see the drawing of some damaged pivots **(Figure 3)**.

Table 3. The number of the 2nd stage juveniles (J2) of CCN found on the infested wheat pivots in 2006 cropping season with the approximate area of infestation at each pivot.

Pivot	A3P19	A6P14	A7P15	A7P16	B5P15	D3P5	D4P8
Number of 2nd stage juveniles per liter of soil	5920	1632	2640	5864	36580	16800	7040
Approximate damaged area (Ha.)	0.4	0.0025	0.20	0.15	1.7	0.01	0.005
Pivot	**F3P5**	**G3P6**	**J21P23**	**J41P81**	**J45P83**	**K2P6**	**K6P15**
Number of 2nd stage juveniles per liter of soil	9600	33520	22930	39040	4480	5976	7412
Approximate damaged area (Ha.)	0.005	0.005	0.40	0.50	0.0025	0.01	0.150

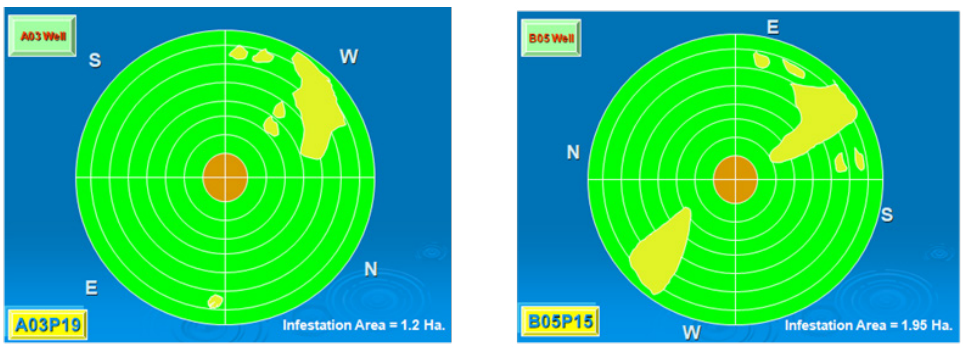

Figure 3. (3a & 3b) Drawing of the CCN yellow patches on wheat pivots in 2007.

The analysis results on soil samples collected on November 2006 from 10 infested pivots before sowing new crop for 2007 season are presented in **Table 4**. The results had shown 6 pivots are with the number of eggs per gram of soil were above the threshold level (5 eggs/gram of soil), and two pivots were with density near the threshold level.

Table 4. Analysis results of soil samples of ten pivots on November 2006 for the presence of CCN before wheat sowing.

Specific Location	Number of Cysts/100 gram Soil	Number of Eggs/Cyst	Number of Eggs/gram of Soil
A07P15 – Over Hang North	7	11	0.77

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A07P16 – Over Hang South	19	36	6.84
A03P19 – T7	15	35	3.25
B05P15 – T7 East	23	68	13.64
B05P15 – T7, T6 West	50	72	36.00
G03P06 – T6 West	6	35	2.10
D03P05 – T7 South West	27	23	6.21
D4P8 -T7 East	4	112	4.48
F3P5 - T7 East	16	69	11.00
K2P6 - T7 East	28	46	12.80

The number of pivots planted with wheat in 2007 was 142 pivots with a total area of 7165 Ha.; 15 pivots were found infested with CCN nematode in small batches at most of the towers areas and the infested areas expanded with farm machinery **(Figure 4)**. The estimated infested areas were 5.039 hectares. The infested pivots were: A03P19, A06P14, B05P15, F03P05, D03P05, J41P81, C04P17, G02P04, J32P66, J29P02, J28P05, K02P06, K03P04, K06P15, and G03P06.

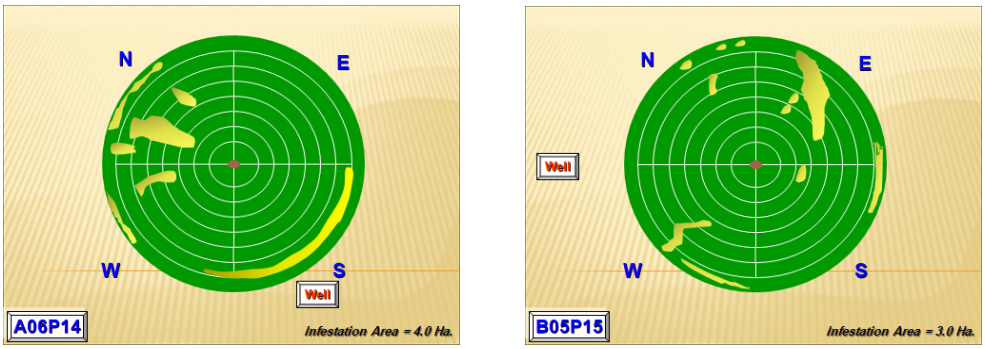

Figure 4. (Fig 4a & 4b) Drawing of the CCN yellow patches on wheat pivots in 2008.

The number of pivots planted with wheat in 2008 was 139 pivots with a total area of 6648 Ha. 43 pivots were found infested with CCN nematode in relatively larger batches than earlier years in some pivots at most of the towers areas as the infested areas expanded more over this year in comparison to earlier years through farm machinery. The estimated infested areas were 21.071 hectares. The infested pivots were: B05P15, C04P17, G02P04, G03P06, G04P08, A06P14, A04P25, D03P05, D04P08, F03P05, J20P26, J29P02, J41P81, K02P06, K03P04 K06P15, C06P17, C09P07, C07P05, J29P01, E06P02, G01P01, G02P03, G04P09, A02P21, A05P11, A06P13, A12P07, A07P15, J28P06, J36P76, F18P36, J11P56, D04P07, J01P37, J05P45, J19P28, J21P22, F11P22, J15P73, J33P65, J14P62, and J31P71.

Impact on wheat crop infested with CCN, showing the level of infestation and the yield in three respective cropping seasons 2006, 2007 & 2008 is represented in **Table 5**, the summary of the results is represented in the **Table 6**. Under the integrated CCN management plan, the total area of infestation was 3.78 Ha in 2006 and it increased slightly to 5.039 Ha in 2007; in 2008 it increased to 21.071 Ha. and these figures correspond to 0.05%, 0.07%, and 0.32% of the total wheat area of each year respectively. Reduction in the yield of infested pivots compared to the mean production in 2006, 2007 and 2008 reached 13.52%, 12.76%, 2.31% respectively. However, %reduction in the mean production of infested pivots in comparison to the general mean production in 2005 which reached 8.098 M.T./Ha. when we did not observe CCN infestations were 14.67%, 13.81%, 7.76% respectively.

Table 5. Survey results of some infested wheat pivots showing the level of infestation with CCN combined with the yield on three cropping seasons.

S.N.	Pivot	Density of J2/L Soil & Yield M.T. /Ha in 2006.	Density of J2/L Soil & Yield M.T. /Ha. in 2007	DensityJ2/L Soil& Yield M.T. / Ha in 2008.
1	A02P21	No	No	480 (6.53)
2	A03P19	5920 (7.21)	13620 (6.66)	No
6	A06P14	1632 (7.67)	35340 (6.59)	3720 (5.83)

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10	B05P15	36580 (8.11)	12028 (7.10)	6000 (6.75)
11	C04P17	No	10204 (8.15)	10080 (7.58)
12	C06P17	No	No	22800 (7.50)
15	D03P05	16800 (8.41)	6384 (7.62)	8400 (6.51)
16	D04P07	No	No	480 (7.94)
17	D04P08	7040 (7.37)	No	4560 (6.62)
18	E06P02	No	No	840 (7.78)
19	F03P05	9600 (7.41)	8824 (7.96)	4560 (7.67)
20	F11P22	No	No	240 (8.08)
21	F18P36	No	No	1320 (7.75)
24	G02P04	No	2420 (8.53)	6640 (7.66)
25	G03P06	33520 (8.4)	9200 (8.01)	3400 (8.08)
34	J20P26	No	No	8160 (7.46)
35	J21P22	No	No	3360 (7.75)
36	J21P23	22930 (7.05)	No	No
37	J28P05	No	4236 (7.45)	No
39	J29P01	No	No	14400 (7.42)
40	J29P02	No	2232 (6.97)	7600 (7.02)
45	J41P81	39040 (6.99)	8680 (7.24)	20400 (7.05)
46	K02P06	5976 (8.05)	5700 (7.12)	2880 (7.07)
47	K06P15	7412 (7.52)	3694 (7.33)	2520 (7.92)

N.B: No means No Wheat planting.

Table 6. Summary survey results of infested wheat pivots showing the level of infestation with CNN combined with the yield in three cropping seasons 2006-2008.

Comparison Item	Year of Production		
	2006	2007	2008
Number of Infested Pivots	14	15	43
Damaged Area Ha	3.78	5.039	21.071
Percentage Damaged Area	0.05%	0.07%	0.32%
Wheat Pivots General Yield M.T/Ha	7.991	8.001	7.647
Damaged Wheat Pivots Yield M.T/Ha	6.91	6.98	7.47
% Yield Reduction Relative to each year	13.52	12.76	2.31
% Yield Reduction Relative to 2005*	14.67	13.81	7.76

*Mean wheat pivots yield in 2005=8.098 M.T./Ha

The results of wheat productivity under different crop rotation is represented in the **Table 7**. The productivity of wheat crop increased after two-years crop rotation with alfalfa; wheat yield increased in the absence of CCN infestations and ranged 10-22.7% which is higher than on the pivots infested with CCN which increased by 2.4-12.7%. The productivity of wheat crop slightly increased by 0.4% after one-year crop rotation with potato in the absence of CCN infestations.

Table 7. Effect of CCN and crop rotation with different crops on the yield of wheat

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Pivot	Cropping Season				
	2007	2008	2009	2010	2011
J32P66 No Infestation % Increase	Wheat 8.10 +1.24%	Alfalfa --	Alfalfa --	Wheat 8.095 +10.03%	Wheat 8.790 +16.42%
J31P70 No Infestation % Increase	Alfalfa --	Alfalfa --	9.264 +11.68%	8.252 +1207%	8.590 +13.77
J5P45 No Infestation % Increase	Potato	7.73 +0.4%	Alfalfa	8.511 +15.69%	9.264 +22.7%
J28P5 0.05 Ha % Increase	Wheat 7.450 - 6.81%	Alfalfa	Wheat 8.550 +3.07%	Wheat 7.696 +12.70%	Potato
J45P83 0.003 Ha % Increase	Alfalfa	Alfalfa	Wheat 7.696 - 7.22%	Wheat 7.539 +2.47%	Alfalfa
A3P19 1.2 Ha % Increase	Wheat 6.660 -16.76%	Idle	Onion	Wheat 7.749 +5.33%	Wheat 6.750 - 10.6%
A5P11 0.01 Ha % Increase	Wheat 6.300 -16.76%	Wheat 6.87 -10.16%	Onion	Wheat 5.265 - 28.44%	Wheat 6..97 - 7.68%
D4P8 0.005 Ha % Increase	Wheat 7.480 - 6.51%	Wheat 6.619 - 13.44%	Onion	Wheat 6.878 - 6.51%	Wheat 6.96 - 7.8%
G1P1 0.005 Ha % Increase	Wheat 7.600 5.01%	Wheat 7.070 +7.55%	Wheat 5.770 - 30.44%	Wheat 6.251 - 15.03%	Wheat 6.142 - 18.6%
G2P4 0.005 Ha % Increase	Wheat 8.530 6.61%	Wheat 7.660 +0.17%	Wheat 7.100 - 14.41%	Wheat 6.549 - 10.98%	Wheat 6.474 - 14.25%
Number of Wheat Pivots	142 (7165 Ha)	139 (6648 Ha)	129 (5035 Ha)	135 (6569 Ha)	128 (5655 Ha)
Wheat Pivots Mean Yield M.T/ Ha	8.001	7.647	8.295	7.357	7.550

The productivity of wheat crop after one-year crop rotation with onion was decreased by 6.51-28.44% in two pivots and increased by 5.33% in one pivot in the presence of old CCN infestations. Continuous cropping wheat without following crop rotation decreased productivity by 10.98-30.44%.

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Wheat pivots infested with CCN led to increased infestation with ryegrass & sometimes downy brome which replaced weak plants damaged by the CCN nematode, and both grasses are resistant to wheat herbicides. The effect of the infestations with the CCN & grasses mainly ryegrass on the yield of the wheat crop was investigated in 2009 on five wheat pivots: G2P4, G3P6, C4P17, J41P81 and F17P34. Detailed data are shown in **Table 8**, and summary of the results are presented in **Table 9**. Analysis results on February 20, 2009 showed High numbers of 2nd stage juveniles (J2) per liter of soil on all pivots except F17P34 with low numbers 1440 J2's. The number of eggs/gram of soil was high at C4P17 & G3P6 which reached 31.62, 25.3 eggs gram of soil respectively, and it was with medium level 14.26 eggs/gram of soil at J41P81; the number of eggs per gram of soil at G2P4 and F17P34 was near the threshold level and it reached 4.97, 6.62 eggs/gram of soil respectively. So, the level of CCN inoculums was above the threshold level on all of the five pivots on Feb 20, 2009. Ryegrass infestations were high on all pivots except at F17P34 was with a medium infestation on a small area. G3P6 had high ryegrass infestation in a smaller area than the other three pivots. Downy brome was observed at G2P4 along with ryegrass. High yield reduction occurred in the presence of high infestations with both CCN & grasses, and as the density of CCN & grasses was increased yield reduction was increased as observed at J41P81 and C4P17 with yield reduction 42.43, 30.26% respectively. High ryegrass infestations and low CCN density caused 21.05% yield reduction at G2P4, while at G3P6 reduction reached 4.5% with a limited infested area of both. No reduction in the yield occurred in the presence of low infestation with CCN and ryegrass at F17P34 with productivity 8.298 M.T./Ha. Final population density with CCN deteriorated at G2P4, G3P6 & C4P17 which were with high ryegrass infestation as the final population at these pivots on May 2009 was less than the initial population on Feb 2009.

Table 8. Number of different stages of the CCN in soil samples collected on Feb 20, 2009 from five wheat pivots infested with grasses (Data are means of 3 replicates).

Pivot	Number of 2nd Stage Juveniles/Liter of Soil	Number of Cyst/100 gram of Soil	Number of Eggs/Cyst	Number of Eggs/Gram of Soil	Yield M.T./ Ha	Final Population Eggs/Gram of Soil	Grass Infestations on May 2009
G02P04	4320	26.3	18.9	4.97	6.549	3.9	High Rye Grass Medium Brome Grass
G03P06	8713	76.89	32.9	25.3	7.922	7.6	High Rye grass Infestation, Small area
C04P17	7960	128	24.7	31.62	5.785	5.2	High ryegrass large area
J41P81	6360	156.7	9.1	14.26	5.430	25.8	High ryegrass at T3-T7 (large area)
F17P34	1440	29.68	22.3	6.62	8.298	13.7	Medium ryegrass infestations, small area

Table 9. Summary for the effect of CCN and grasses infestations on wheat yield.

Pivot	G2P4	G3P6	C4P17	J41P81	F17P34
Number of 2nd stage Juveniles per liter of Soil	4320	8713	7960	6360	1440
Number of Eggs/gram of soil	4.97	25.3	31.62	14.26	6.62
Area Infested with CCN (Ha.)	1	0.05	2.5	1.5	0.005
Yield M.T./Ha	6.549	7.922	5.785	5.430	8.298
% Yield Reduction*	21.05%	4.5%	30.26%	42.43%	No effect
Final Population Eggs/ gram of Soil	3.9	7.6	5.2	25.8	13.7

*General Average Yield in 2009 = 8.295 M.T./Ha.

The results of testing 29 wheat genotype seeds samples (varieties) of the 3rd Elite Bread Wheat (3rd EBWYT) for resistance to CCN in comparison to the existing wheat cultivar Yecora Rojo is represented in the following. The mean number of cysts per 100 grams of soil is shown in **Table 10** and **Figure 5**. The number of eggs/gram of soil at the start of the trial was 4.88 eggs/gram of soil. All of the wheat varieties were susceptible to the CCN; Yecora Rojo (Y.R.) was more susceptible than all of the wheat varieties except variety # 24 which produced more cysts 37 cysts. Wheat variety # 15 produced 12.7 cysts which is the lowest number of cysts/100 gram of soil in comparison to the other varieties followed by variety # 9 and # 28 which they produced 17.7, 18 cysts. It appears that variety # 15 is resistant to the CCN population as the calculated number of new cysts is 1.8: Final Number of Cysts -

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Initial Number of Cysts=12.7 - 10.9=1.8 cyst/100 gram soil. Statistical analyses using Statistix9 Software is represented in **Table 10** have shown no significant difference between the varieties for the number of cysts/100 gram of soil.

Table 10. Statistical analysis using Stastistix9 Software for the number of cysts / 100 gram of soil

Randomized Complete Block AOV Table for Number of Cysts per 100 Gram of Soil

Source	DF	SS	MS	F	P
Blocks	2	38.07	19.0333		
Variety	29	2387.43	82.3253	1.14	0.3292
Error	58	4190.6	72.2517		
Total	89				

Note: SS are marginal (type III) sums of squares

Grand Mean 24.100 CV 35.27

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	24.72	24.7204	0.34	0.5631
Remainder	57	4165.9	73.0856		

Relative Efficiency, RCB 0.98

Means of Number of Cysts per 100 Gram of Soil for each Variety

Variety	Mean	Variety	Mean	Variety	Mean
1	33.667	11	25.667	21	24.667
2	22.333	12	26	22	24.333
3	23.333	13	28	23	24
4	22	14	33	24	37.333
5	19.667	15	12.667	25	20
6	22.333	16	26.667	26	26
7	27	17	30.667	27	19.667
8	19.667	18	24.667	28	18
9	17.667	19	28	29	25.667
10	18.667	20	20	30	21.667

Observations per Mean 3

Standard Error of a Mean 4.9075

Std Error (Diff of 2 Means) 6.9403

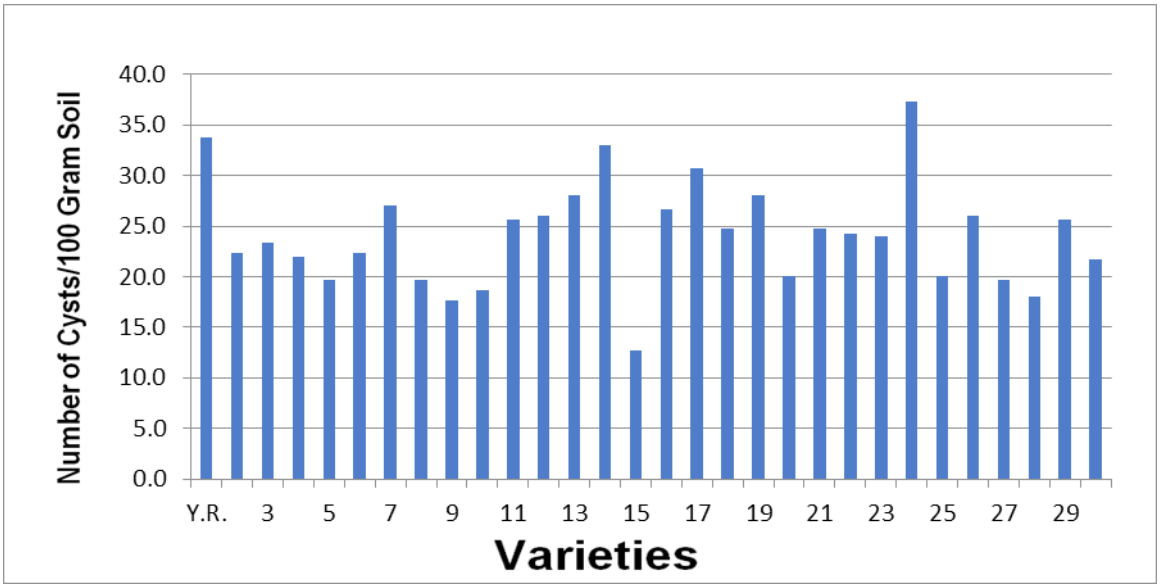

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Figure 5. The number of CCN cysts/100 gram of soil produced on 30 wheat varieties.

The mean number of eggs per cyst of CCN is shown in **Table 11** and **Figure 6**. The number of eggs/cyst on all of the wheat varieties was relatively low, but they are similar to TADCO field's survey records. Yecora Rojo produced more eggs/cyst than all of the other wheat varieties except variety # 12 & 30 which produced more eggs/cyst: 56, 55 eggs respectively. Wheat variety # 28 produced 15.7 eggs/cyst which was the lowest number followed by variety # 10 and # 23 which produced 18. 18.7 eggs respectively.

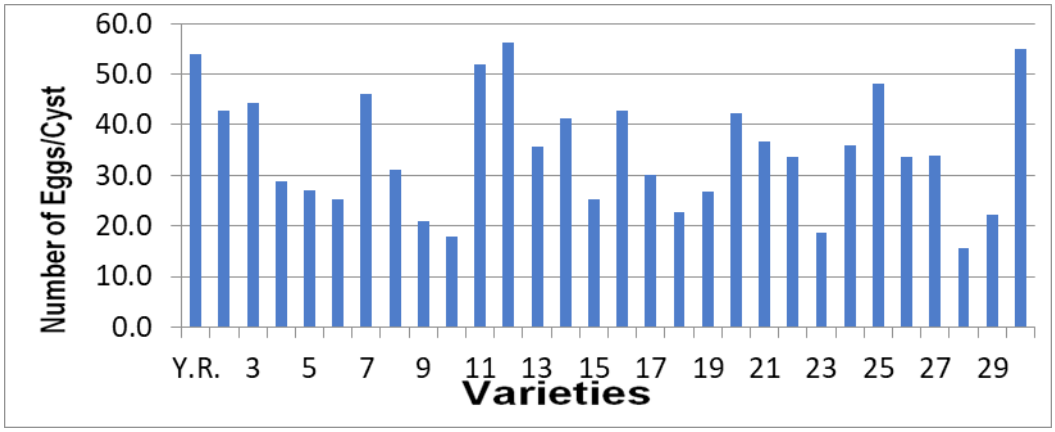

Figure 6. The number of Eggs/Cyst of the CCN produced on 30 wheat varieties.

Statistical analyses using Statistix9 Software is represented in Table 11 have shown no significant difference between the varieties for the number of eggs/cyst.

Table 11. Statistical analysis using Stastistix9 Software for the number of eggs/cyst

Randomized Complete Block AOV Table for Number of Eggs per Cysts

Source	DF	SS	MS	F	P
Blocks	2	241.5	120.744		
Variety	29	11824.9	407.755	1.4	0.1397
Error	58	16952.5	292.285		
Total	89				

Note: SS are marginal (type III) sums of squares

Grand Mean 34.889 CV 49.00

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	82.0	81.989	0.28	0.6007
Remainder	57	16870.5	295.974		

Relative Efficiency, RCB 0.99

Means of Number of Eggs per Cysts for each Variety

Variety	Mean	Variety	Mean	Variety	Mean
1	54	11	52	21	36.667
2	42.667	12	56.333	22	33.667
3	44.333	13	35.667	23	18.667
4	28.667	14	41.333	24	36
5	27	15	25.333	25	48
6	25.333	16	42.667	26	33.667
7	46	17	30	27	34
8	31	18	22.667	28	15.667
9	21	19	26.667	29	22.333
10	18	20	42.333	30	55

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Observations per Mean 3

Standard Error of a Mean 9.8706

Std Error (Diff of 2 Means) 13.959

The mean number of eggs per gram of soil is shown in **Table 12** and **Figure 7**. Wheat varieties # 28, 15, 10, 23, 5, 6 produced 3.06, 3.1, 3.8, 4, 4.7 and 4.8 eggs/gram of soil which were less than the numbers at the start of the trial 4.88 eggs/gram of soil which were less than the numbers at the start of the trial 4.88 eggs/gram of soil. In comparison wheat varieties # 12, 24, 14 produced the highest numbers of eggs/gram soil 14.6, 13.9, 13.5; Yecora Rojo produced 12.2 eggs/gram of soil. Statistical analyses using Statistix9 Software is represented in **Table 12** have shown no significant difference between the varieties for the number of eggs/gram of soil.

Table 12. Statistical analysis using Stastistix9 Software for the number of the number of eggs/gram of soil

Randomized Complete Block AOV Table for Number of Eggs per Gram of Soil

Source	DF	SS	MS	F	P
Blocks	2	42.46	21.2308		
Variety	29	1080.77	37.268	1.04	0.4426
Error	58	2087.03	35.9833		
Total	89				

Note: SS are marginal (type III) sums of squares

Grand Mean 8.7511 CV 68.55

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	0.97	0.9659	0.03	0.8715
Remainder	57	2086.07	36.5976		

Relative Efficiency, RCB 0.99

Means of Number of Eggs per Gram of Soil for each Variety

Variety	Mean	Variety	Mean	Variety	Mean
1	12.2	11	13.2	21	10.1
2	10	12	14.6	22	7.6
3	12.8	13	10.2	23	4
4	6.2	14	13.467	24	13.9
5	4.7	15	3.1	25	9.3
6	4.8	16	13.1	26	8.8
7	12.7	17	8.8	27	7
8	6.2	18	5.1	28	3.067
9	6	19	7.5	29	10.5
10	3.8	20	9.9	30	9.9

Observations per Mean 3

Standard Error of a Mean 3.4633

Std Error (Diff of 2 Means) 4.8978

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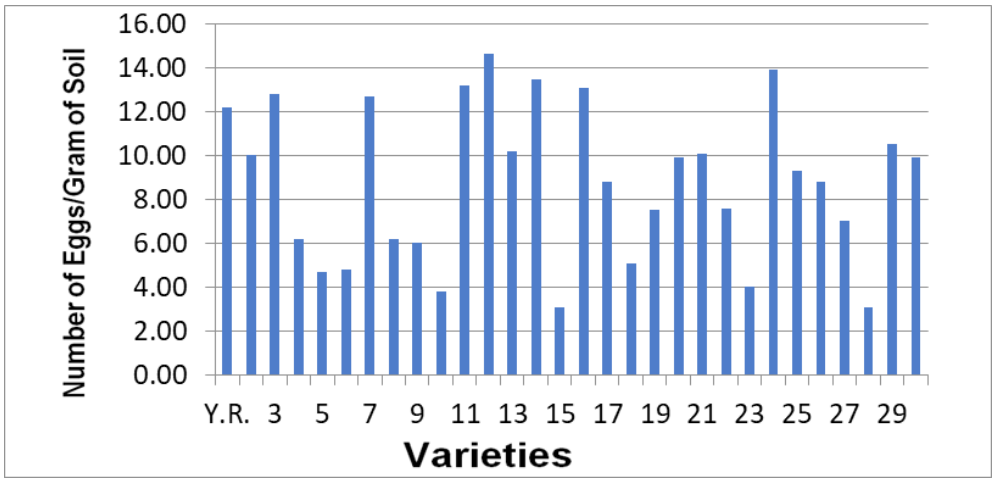

Figure 7. The number of Eggs/Gram of soil of the CCN produced on 30 wheat varieties.

DISCUSSION

Tabuk Agric. Dev. Co. (TADCO is one of the leading Saudi companies on crop production of field crops & vegetable crops under Centre pivot irrigation system in addition to the production of fruit crops, grapes and olives. Due to its geographic location on the northwest of Saudi Arabia with semi Mediterranean climate, high water quality, professional irrigation management and skilled labors, TADCO achieved high yields and high quality of wheat grains during the period 2001-2011, and in 2003 average wheat production of an area over 5000 Ha reached 8.73 M.T./Ha; TADCO also specializes in the production of certified wheat seeds. To achieve a high yield of wheat, the growers at TADCO prefer to sow the wheat seeds in this area during the period Mid December - Mid January every year. Unfortunately, the weather conditions during this period are favorable for cereal cyst nematode (CCN) infections. CCN infestations were prevalent in wheat growing areas of Saudi Arabia such as Al-Kharj, Gassim & Hael [5,6], and the estimation of yield reduction ranged 40 - 92% on heavily infested sandy soil in these areas especially under monocropping system [2].

In this study, the number of CCN hatched juveniles into the soil samples taken from the yellow patches of stunted wheat plants at the early stage of seedling growth were utilized as a tool to evaluate the degree of infestation and the expected damage by the CCN on each wheat pivot as the level of damage is affected by the juveniles numbers (J2) in the soil and the area of infestation [10]. The method of extraction of the juveniles from the soil samples by the sieving & centrifuge method with sugar floatation [7] is a rapid method, accurate and easy to conduct in a short period of time.

To prevent the expected heavy losses on wheat yield, an integrated cereal cyst nematode management plan was implemented and executed into short term & long-term management plans as mentioned under the materials & methods. Under the CCN management plan and under the growing conditions at TADCO, reduction in the yield of CCN infested pivots was minimized to 14.67% in 2006, 13.8% in 2007 and 7.76% in 2008 in comparison to the general wheat yield in 2005 with an average 8.098 M. T./Ha. When we did not observe CCN infestations as the damaged areas were treated with systemic granular nematocide and top dressing with granular NPK 14-38-10 and granular potassium sulphate fertilizers.

Two years crop rotation with alfalfa to sow wheat seeds using the Rapid Vaderstad No-Till planter, was very effective as a CCN control measure since wheat productivity was increased by 2.4-12.7% and crop rotation with alfalfa on pivots free from CCN led to higher yield increase ranged 10-22.7%. Actually, the average yield of wheat crop on most of the alfalfa rotation pivots ranged 8-9 M.T./Ha which alleviated yield reduction caused by the CCN. This increase was due to pivots being clean from grasses and CCN in addition to increased soil fertility after alfalfa crop rotation. Productivity after one-year crop rotation with onion reduced yield by 5-28.4% due to the spread of ryegrass resistant to herbicides.

Continuous wheat cropping without following crop rotation led to increased wheat yield reduction due to increased infestations with CCN, and yield reduction was in the range of 10.98-30.44%, which is partially in agreement with research results in Al-Kharj in Saudi Arabia with crop loss 40-92% [2]. These results are also in agreement with reports from different countries with similar climatic conditions of Saudi Arabia during wheat crop development [4,11,12]. For example, wheat crop loss in Turkey is 4.3-25.7%, 15-20% in Pakistan, 23-50% in Australia.

Evaluation of wheat crop productivity at TADCO farm over the period 2006-2011 with an average 7.77 M.T./Ha. had shown a minimum yield reduction around 4.1% in comparison with the yield obtained in 2005 (8.098 M.T./Ha). These results proved a great degree of success of the CCN integrated management in minimizing the expansion of the infested areas with the CCN and minimizing yield losses. In addition, control of herbicides resistant grasses such as ryegrass & downy brome during alfalfa rotation. Wheat productivity stability at TADCO was due to the implementation of CCN integrated management through two years

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crop rotation with alfalfa on infested pivots, the application of granular systemic nematocide, top dressing of NPK 14-38-10 & potassium sulphate granular fertilizers on all of the wheat pivots including the damaged areas which increased the tolerance level of the wheat crop to the CCN invasion. It is well known that potassium nutrient raises the level of plants resistance to sucking insects, some foliage diseases & root-knot nematodes. Similar results were obtained by [13-15] that the application of certain NPK, urea fertilizers and nematocides had led to an increased dry weight of infected wheat plants due to their effect on the number of cysts/root system.

In the field survey study, we observed a very high number of J2's near the roots at the seedling stage, so this caused severe damage to wheat plants. The number of the cysts produced by the end of the season was relatively low as shown on the soil survey records of infested pivots before sowing new wheat crop which ranged 27-50 cysts/100 gram and the number of eggs/cyst ranged 68-112 eggs/cyst and this is very low in comparison to cold temperate areas which is normally 200-250 eggs/cyst [8]. By the end of the season, the number of eggs/gram of soil ranged 10-36 eggs/gram of soil, so the multiplication rate of the CCN at TADCO was in the range of 2.5-4.1 which is far less than the multiplication rate in the mid of Saudi Arabia which reached 5.4-10.05 [2]. Field records in 2009 showed deterioration of the CCN population density on the pivots infested with ryegrass as the final population was less than the initial density since ryegrass is not a good host for the CCN besides it affects the wheat as a suitable host for the CCN.

The results on screening bread wheat germplasm on pots (black plastic bags) experiment showed that variety (entry) # 15 produced 1.8 cysts/100 gram of soil an indication that it is resistant to CCN. Also, varieties with the entries # 28, 15, 10, 23, 5, 6 showed some degree of resistance to CCN as they produced 3.06, 3.1, 3.8, 4, 4.7 and 4.8 eggs/gram of soil which were less than the number of eggs/gram of soil at the start of the trial 4.88 eggs/gram soil. The number of eggs/gram of soil produced by the varieties is an important parameter in determining resistant varieties as the resistant varieties produce less number of eggs/gram of soil than the numbers at the start of the trial. These results can be useful for future development of resistant wheat cultivars for the CCN in Saudi Arabia.

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