



COMPARATIVE OF FUNGICIDAL EFFICACY AGAINST YELLOW RUST DISEASE IN WHEAT PLANTS IN COMPATIBILITY WITH SOME BIOCHEMICAL ALTERATIONS

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Received: Jan. 23, 2019

Accepted: Dec. 15, 2019

ABSTRACT: *Yellow rust of wheat caused by Puccinia striiformis is a destructive disease of wheat. It is present in almost all the wheat growing areas of the world and is a formidable threat to wheat production. The use of chemical fungicides to control plant diseases is an integral component of crop management but should be safety to humans and the environment. Controlling yellow rust disease of wheat by using eight fungicides (Tilt[®], Fungshow[®], Abatchi[®], Crwan[®], Sumi-8[®], Punch[®], Emnent[®] and Meanar[®]) in wheat spray treatments was studied. Residue analysis of such fungicides in grain and straw of wheat after harvest was carried out. The present investigations were carried out in two different locations during the year of 2017/18 growing season and the results of tested fungicides revealed that minimum disease severity of 1.67 % was recorded with Tilt[®] 25% EC. This treatment gave a disease control of 98.07 %, thus increasing the grain yield and 1000 kernel weight by 29.92 and 73.61 %, respectively. It was followed by a disease control of 96.91 % achieved with Crwan[®] 25% EC which gave an increase of 24.39 and 69.34 % in grain yield and kernel weight, respectively. The analysis of total protein and carbohydrates in grains and straw samples of treated plants shown significant differences within all treatments. Data indicated the residue analysis of fungicides in grain and wheat straw exceeded the allowable maximum residue limits (MRLs) value when using fungicides as spray treatment comparing the MRLs on international authorities.*

Key words: *Wheat, Yellow rust, Puccinia striiformis, Disease control, Fungicide residues.*

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops and consumed food crops in the world. The high starch content in the grain (60-70%) is considered the main source of calories for the human diet (Shewry, 2009). It has an important element in agricultural economy for many countries around the world. It is planted in the largest areas followed by maize, barley

and sorghum (FAO, 2009). Yearly, wheat is cultivated on 224.53 million hectares of land, yielding for 672.2 million tons in the world (USDA, 2010). According to this report the world average wheat production is 2.99 tons/ha. The production of wheat is effected by various biotic and abiotic agents. Among the biotic agents, wheat rust diseases are causes severe losses in grain yield of wheat all over the world. Yellow rust of

wheat caused by *Puccinia striiformis* f. sp. *tritici* is posing a potential threat to worldwide wheat production owing to their wide distribution (Chen, 2005). The disease can cause huge losses in grain yields due to reduced size and number of kernels (Weise, 1987). Murray *et al.* (1995) found that wheat stripe rust reduced the grain yield with 84%, kernel mass with 43% and kernel number with 72%. Yellow rust can cause 100 % yield losses in case of susceptible varieties if infection started very early and the disease continues to develop during growing season (Afzal *et al.* 2007). Chen (2005) found that depending upon the susceptibility of variety, earliness of infection, rate of disease progress and duration of disease, the yield losses caused by yellow rust ranges between 10-70%. Hasan *et al.* (2016) and Gebril *et al.* (2018) reported that wheat yellow rust disease have affected on grain yield of some Egyptian wheat cultivars and also observed negative correlation between disease severity and yield components.

The use of synthetic compounds to control plant diseases dates back to the 19th century, when in 1807, Prévost demonstrated the control of wheat bunt (*Tilletia caries*) by applying copper sulphate (Russell 2005). The control of stripe rust is very crucial for the global food security. Line (2002) reported that Triadimefon has been used as a foliar fungicide to control yellow rust disease which prevented multi-million dollar losses. Different fungicides like Tilt[®], Quadris[®], Stratego[®], Quilt[®] etc. have been registered for controlling of yellow rust of wheat worldwide (Chen 2007). Viljanen-Rollinson *et al.* (2002) reported that the efficacy of a fungicide depends partly on the growth stage of the crop and disease severity during application. In the view of these concepts, the present study was conducted with the following objectives; (1) to compare the efficacy of different fungicides against stripe rust on wheat

plants. (2) to detect the residue levels in grains and straw of wheat crop in compatibility with some biochemical alterations.

MATERIALS AND METHODS

1. Experimental design and disease assessment:

Bread wheat cultivar Sids 12, highly susceptible to yellow rust disease and planted at two locations; Gemmeiza Agricultural Research Station, El-Gharbiya Governorate and Itay El-Baroud Agricultural Research Station, El-Behira Governorate. Present investigations were carried out during 2017/18 growing season to control yellow rust disease of wheat caused by *Puccinia striiformis*. The trial was sown in 4 x 3 m plots using the variety Sids 12 in a Randomized Complete Block Design with three replicates for each treatment. Proper fertilizer application and control of insect-pests was followed. Highly susceptible cultivar Morocco was sown on the border rows of the experimental area for the early development and spread of disease. It was artificially inoculated during early January to create stripe rust epidemics. Eight fungicides viz. Tilt[®] 25% EC (Propiconazole), Fungshow[®] 12.5% WP (Diniconazole), Abatchi Akonazole[®] 25% EC (Propiconazole), Crwan[®] 25% EC (Propiconazole), Sumi-8[®] 5% EC (Diniconazole), Punch[®] 40% EC (Flusilazole), Emnent[®] 12.5 EW (Tetraconazole), Meanar[®] 41% EC (Cyproconazole + Propiconazole) were used. The fungicides should be sprayed when leaves showed the symptoms of yellow rust and applied twice at 10 days interval (Bal, 2014). Rust severity was determined according to modified Cobb's scale (Peterson *et al.* 1948). Total grain yield (Ardab/Feddan) and thousand kernel weight were also recorded.

2. Analytical procedures

Both grains and straw were grinded until fine powder. Then, 5 g of each was placed into 50 ml polyethylene tube and mixed with 15 ml of acidic acetonitrile. After that, 2 g of magnesium sulphate and 0.25 g of sodium chloride were added, shaken vigorously for 15 minutes and then centrifuged for 10 minutes at 4000 rpm. 1ml of supernatant was transferred into 15-ml centrifuge tube containing 100 mg PSA and 600 mg of magnesium sulphate. The extract was processed as described above. The supernatant was filtered through a 0.2 µm PTFE filter (Millipore, USA) (Jiang *et al.* 2009).

Compounds; diniconazole, propiconazole, tetraconazole and cyproconazole were subjected on gas chromatography instrument (HP Model 5890) equipped with Ni⁶³ electron capture detector (ECD). An analytical column (30 m length×0.32 mm i.d.×0.25 µm film thickness) was used at temperature program as follows: injector (280 °C), oven (220 °C for 2 min and hold to 280 °C after 10 min) and detector (320 °C), respectively and Nitrogen was used as a carrier gas at flow rate 4 ml/min.

Other examined compounds were exposed on High Performance Liquid Chromatography (HPLC) instrument (Agilent 1100 HPLC system, USA). The chromatographic used column was C₁₈ Zorbax XDE (250 mm × 4.6 mm, 5 µm). The run was done at a flow rate 0.8 ml/min of (CH₃CN: H₂O, 65:35 v/v) at room temperature Twenty µl of each analyte was responded on photodiode array detector at 220 nm. All compounds were identified by their retention times compared to recognized standards.

3. Quality assurance

The methods were validated under the optimized conditions by determining limits of detection (LODs) and

quantification (LOQs). The practical protocol was done according to published guide. All used solvents and reagents were in analytical grade. LOD of all compounds was 0.1 ng. On the other hand, recovery experiment for 'Quick Easy Cheap Effective Rugged and Safe' (QuEChERS) technique was done by spiking control samples with recognized amounts of the compounds and the procedures were done as described above. The mean value for all compounds was 98.0±3.0%.

4. Total carbohydrate quantification.

The powder (0.1 g) of grains or straw was mixed with 4% of NaOH and boiled in water bath for 2 hr. After cooling, the samples were centrifuged at 3000 rpm for 10 minutes. An aliquot (0.5 ml) was mixed with the same volume of phenol reagent 5% and 2.5 ml of concentrated H₂SO₄ for 30 min at room temperature. The optical density was measured at wavelength 490 nm against blank on Spectronic 21D (Milton Roy Co. USA). Glucose was used as a standard to calculate the extension coefficient (Dubois *et al.* 1956) and expressed as % of total weight.

5. Total protein quantification

Powder of samples (0.1 g) was extracted by 10 ml of borate buffer (28.63 boric acid+ 29.8 g KCl + 3.5 g NaOH in one liter of distilled water), kept overnight, then centrifuged at 3000 rpm for 10 min., filtered and completed to 10 ml. Protein concentration was estimated according to (Lowry *et al.* 1951) and expressed as mg/g dry weight.

6. Data analysis

Data was exposed to analysis of variance (ANOVA) and Least Significant Difference (LSD 0.05) was used to compare the means for all the variables within the experiment (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

1. Assessment of disease and yield components

Stripe rust disease pressure was very high and excellent disease epidemic that developed to the level of creating significant difference among treatments across the two locations during the growing season.

Generally, results of tested fungicides are listed in Tables 1 and 2 showing that all treatments reduced the severity of yellow rust and increased harvest yield. The level of disease severity was far higher in the untreated plots compared to treated ones. Application of Tilt® 25% EC at rate 0.1%, recorded the minimum disease severity (1.67%) compared with untreated plots (86.67%). Grain yield exhibited increased percentages 29.92% and 73.61% in 1000 kernel weight compared with untreated ones. Crwan® 25% EC at rate 0.1% closely followed, by giving 94.61% control of stripe rust. It achieved increase values; 24.39 and 69.34 % in grain yield and 1000 kernel weight, respectively. In addition, Fungshow® 12.5% WP at rate 0.1%, Meanar® 41% EC at rate 0.1%, Sumi-8®

5% EC at rate 0.1%, Emnent® 12.5% EW at rate 0.1%, and Abatchi® 25% at rate 0.1% effectively reduced the stripe rust severity. These fungicides increased the grain yields with values; 25.63, 22.87, 18.31, 14.92, and 11.74% and reduced the disease severity with values; 96.91, 95.76, 95.38, 96.61 and 94.61%, respectively. Punch® 40% EC at rate 0.1% exhibited the least control values against stripe rust 93.85%. Many investigators presented similar results. For example, Bal (2014), found that the application of Amistar® fungicide, recorded the minimum disease severity compared with untreated plots. Viljanen-Rollinson *et al.* (2002), noted that the fungicide Azoxystrobin was very effective to reduce the rust diseases in wheat when applied as early eradicator. Bagga (2007), found that Amistar® followed by Score® and Bayleton® significantly reduced the leaf rust severity resulting in improved grain yields and grain weight. Jorgensen and Nielsen (1994) reported that most of the ergosterol biosynthesis inhibiting (EBI) fungicides as triazoles gave 98-100% control of stripe rust.

Table 1. Effect of Different Fungicides on Yellow Rust Severity and Area Under Disease Progress Curve (AUDPC) of Tested Wheat Cultivar During 2017/2018 Growing Season.

Sr. No.	Fungicide	Active ingredient	Rate of application	Disease severity (%)	Disease control (%)	AUDPC
1	Tilt® 25% EC	Propiconazole	25 ml/100 l	1.67 ^c ±0.19	98.07	16.33 ^b ±2.43
2	Crwan® 25% EC	Propiconazole	30 ml/100 l	4.67 ^{bc} ±0.38	94.61	50.17 ^b ±1.78
3	Fungshow® 2.5% WP	Diniconazole	15 g/100 l	2.67 ^{bc} ±0.19	96.91	24.00 ^b ±2.91
4	Meanar® 41% EC	Cyproconazole+ Propiconazole	200 ml/100 l	4.00 ^{bc} ±0.00	95.76	40.83 ^b ±0.67
5	Sumi-8® 5% EC	Diniconazole	35 ml/100 l	5.33 ^b ±0.38	95.38	56.00 ^b ±1.17
6	Emnent® 12.5% EW	Tetraconazole	100 ml/100 l	2.67 ^{bc} ±0.19	96.61	28.00 ^b ±2.33
7	Abatchi® 25%	Propiconazole	15 ml/100 l	4.67 ^{bc} ±0.38	94.61	46.67 ^b ±1.35
8	Punch® 40% EC	Flusilazole	19 ml/100 l	3.67 ^{bc} ±0.19	93.85	36.17 ^b ±2.43
9	Untreated control	---	---	86.67 ^a ±1.92	---	1131.67 ^a ±35.64

-Each value is the mean of three treated replicates ±SE. No significant different at the same letters. Application of spray volume designed as 100l/feddan.

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Table 2. Effect of Different Fungicides on Yield Components of Tested Wheat Cultivar During 2017/2018 Growing Season.

Sr. No.	Fungicide	Mean of Yield Components				
		1000-Kernel weight (g)	Increase in 1000-Kernel weight (%)	Grain yield (Ardab/Feddan)	Increase in Grain Yield (%)	Straw yield (Ton/Feddan)
1	Tilt® 25% EC	52.38 ^a ±1.25	73.61	18.80 ^a ±0.52	29.92	4.23 ^{ab} ±0.16
2	Crwan® 25%EC	51.09 ^b ±0.75	69.34	18.00 ^{ab} ±1.05	24.39	4.13 ^{abc} ±0.16
3	Fungshow® 12.5% WP	46.71 ^b ±1.17	54.82	18.18 ^{ab} ±0.64	25.63	4.00 ^{abc} ±0.09
4	Meanar® 41% EC	46.63 ^{bc} ±0.92	54.55	17.78 ^{ab} ±1.32	22.87	3.89 ^{abc} ±0.03
5	Sumi 8® 5% WP	45.17 ^{bc} ±0.62	49.71	17.12 ^{ab} ±0.85	18.31	3.68 ^{bc} ±0.21
6	Emnent® 12.5% EW	44.75 ^c ±0.24	48.32	16.63 ^{ab} ±0.19	14.92	3.60 ^c ±0.07
7	Abatchi® (25%)	42.48 ^c ±0.42	40.80	16.17 ^{ab} ±0.04	11.74	3.53 ^c ±0.14
8	Punch® (40%) EC	41.70 ^d ±0.35	38.21	15.97 ^{ab} ±0.77	10.36	3.51 ^{cd} ±0.09
9	Non- Protected Control	30.17 ^a ±0.15	---	14.47 ^c ±0.05	---	2.92 ^d ±0.01
10	Protected control	54.07 ^b ±1.06	79.11	19.18 ^a ±0.07	32.55	4.47 ^a ±0.05

-Each value is the mean of three treated replicates ±SE. No significant different at the same letters.

Fungicide efficacy is depend on proper application timing to achieve optimum effectiveness of it as detected by labeled instructions and disease level in the field at the time of application. Kiersten (2016) reported that the differences in efficacy among fungicide products were detected by direct comparisons among products in field trials and are depend on a single application of the labeled rate. The popularity of spraying fungicides to control plant diseases; returns of up to three times the cost involved often were obtained from fungicide application (Ordish and Dufour 1969). Cook and King (1984) conducted experiments from 1978 to 1982 in the United Kingdom, and noted that applying pesticides to winter wheat resulted in a yield response of up to 89%, and the value of increased yield from pesticide application to cereals in 1982 was nearly double the pesticide costs. Jørgensen *et al.* (2000) noted that the fungicide application to control wheat

powdery mildew and *Septoria* diseases resulted in grain yield increases of 400-2700 kg/ha with margin of cost varying from 500 kg/ha to 2000 kg/ha. Wegulo *et al.* (2009) noted that up to 42% yield loss was stopped by applying foliar fungicides to winter wheat. Kelley (2001) noted that for the period of six years, propiconazole fungicide significantly increased winter wheat yield up to 77%. Vamshidhar *et al.* (1998) observed significant yield increases from fungicide usage against disease complex of leaf rust, tan spot, and *Septoria tritici* blotch in wheat. Ransom and McMullen (2008) noted that within an environment and across wheat varieties, fungicides promoted yields in range; 5.5 to 44.0%. Milus (1994) reported that the application of tebuconazole and propiconazole at Zadoks growth stage (GS) 37 (Zadoks *et al.* 1974) followed by triadimefon + mancozeb at GS 55 against leaf rust and *Septoria tritici* blotch consistently

resulted in low disease severities and high wheat yield.

2. Residue levels.

The residue levels of examined fungicides in grains and straw of wheat plants were determined after harvest. The residue levels in grains were greater than those in straw samples. Meanar[®] (cyproconazole) exhibited the greatest concentration in grain samples (2.201 mg/kg), but was found in below detection limit in straw samples. Eminent-treated plants exhibited the least concentration of tetraconazole (0.016 mg/kg) in grain samples and were below detection limits in straw samples. The residue levels of these compounds were as follows: Meanar[®] > Tilt[®] > Sumi-8[®] > Punch[®] > Fungshow[®] > Abatchi[®]. Other compounds did not exceed 0.036 mg/kg. Fungshow[®] and Punch[®]-treated plants exhibited concentrations in straw greater than those in grain samples accounting mean values of 0.653 and 0.705 mg/kg, respectively. Sumi-8[®] exhibited the mean value; 0.531 mg/kg in straw samples followed by Abatchi[®] (0.289 mg/kg). All examined fungicides exhibited concentration upper rough of EU permissible limits (European Commission), except tetraconazole which was found down MRL (0.1 mg/kg) (Table 3).

3. Biochemical responses

The examined compounds induced alterations in total carbohydrates and protein in grains and straw samples of treated plants (Table 4). In grain samples, Sumi-8[®], Fungshow[®], Tilt[®], Crown[®] and Abatchi[®] enhanced carbohydrates content to be greater than untreated group with mean values; 611.93, 619.65, 537.89, 758.99 and 599.30 mg/g mass, respectively. However, other compounds altered carbohydrates with mean values; 383.51, 376.79, and 298.6 mg/g mass for Punch[®], Eminent[®] and Meaner[®], respectively. In straw samples, all compounds altered carbohydrates content, except Eminent[®] and Meaner[®] induced an increase with values; 878.94 and 808.07 mg/g mass, respectively. Regarding total protein, all compounds induced an increase, except Fungshow[®] and Punch[®] altered protein content with mean values; 9.49 and 10.55 mg/g mass compared with untreated plants (11.64 mg/g mass). However, they altered protein content in straw samples, except Punch[®] and Tilt[®] which increased protein levels to be 29.16 and 27.25 mg/g mass, respectively. Meaner[®] and Abatchi[®] induced the least level (13.87 mg/g mass) compared with untreated group (21.89 mg/g mass).

Table 3. Fungicide Residues (mg/kg) in Grains and Straw of Treated Wheat.

Sr. No.	Fungicide	Active ingredient	Residues (mg/kg)		*MRL (mg/kg)
			Grains	Straw	
1	Sumi-8 [®]	Diniconazole	0.630±0.053	0.531±0.060	0.01
2	Fungshow [®]	Diniconazole	0.436±0.004	0.653±0.037	0.01
3	Tilt [®]	Propinconazole	0.987±0.043	0.708±0.062	0.02
4	Crown [®]	Propinconazole	0.030±0.000	0.033±0.005	0.02
5	Meaner [®]	Propinconazole	0.036±0.001	0.015±0.005	0.02
6	Abatchi [®]	Propinconazole	0.280±0.003	0.289±0.004	0.02
7	Punch [®]	Flusilazole	0.573±0.079	0.705±0.058	0.01
8	Eminent [®]	Tetraconazole	0.016±0.010	BDL	0.1
9	Meaner [®]	Cyproconazole	2.201±0.029	BDL	0.1
LSD 5%		-	0.615	0.487	-

* MRL= maximum residue limits; setting by EU pesticides database and BDL=below detection limit

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Table 4. Total Carbohydrates and Protein in Treated Wheat.

Sr. No.	Fungicide	Total carbohydrates (mg/g mass)		Total protein (mg/g mass)	
		Grains	Straw	Grains	Straw
1	Sumi 8®	611.93 ^c ±0.08	583.51 ^{cd} ±0.14	12.37 ^{cde} ±0.15	17.99 ^d ±0.05
2	Fungshow®	619.65 ^c ±0.08	776.14 ^{ab} ±0.13	9.49 ^e ±0.20	15.16 ^e ±0.09
3	Tilt®	537.89 ^c ±0.09	769.12 ^{ab} ±0.14	12.98 ^{cd} ±0.14	27.25 ^a ±0.05
4	Punch®	383.51 ^{de} ±0.13	671.39 ^{bc} ±0.16	10.55 ^{de} ±0.18	29.16 ^a ±0.05
5	Eminent®	376.79 ^{de} ±0.13	878.94 ^a ±0.12	16.65 ^{ab} ±0.11	19.14 ^{cd} ±0.07
6	Crwan®	758.99 ^b ±0.07	722.96 ^{bc} ±0.14	12.36 ^{cde} ±0.15	21.05 ^{bc} ±0.06
7	Meaner®	298.60 ^c ±0.17	808.07 ^{ab} ±0.13	14.13 ^{bc} ±0.13	13.87 ^e ±0.10
8	Abatchi®	599.30 ^c ±0.08	464.49 ^d ±0.23	17.20 ^{ab} ±0.11	13.87 ^e ±0.10
9	Untreated control	423.16 ^{de} ±0.12	774.03 ^{ab} ±0.14	11.64 ^{cde} ±0.16	21.89 ^{bc} ±0.06

Many investigations relied that most of triazole fungicides decreased growth parameters of crops. For example, tebuconazole decreased seed protein content of Legumes (Ahmed 2011). Other investigations (Guttieri *et al.* 2001; Ceseviciene and Masauskiene 2008; Triboi *et al.* 2003; Souza *et al.* 2004) noted that fungicide efficacy on grain quality based firstly on weather conditions being conducive to the occurrence of fungal diseases and then on fungicide applied. Kelley (2001) found that, fungicides help to keep vegetation of the leaves allowing conditions for larger grains growth and get better quality. Gaurilcikiene *et al.* (2010) reported that the protein levels in harvest grain of fungicide-treated plants were 138 g/kg and 136 g/kg for Rombus® (trifloxystrobin + propiconazole) and Opera® (pyroclostrobin + epoxyconazole), respectively. However, Tilt® treatment exhibited 144 g/kg.

Conclusions

The results of this study revealed that the use of fungicides e.g. Tilt® 25% EC, Crwan® 25% EC, Fungshow® 12.5% WP, Meaner® 41% EC, Sumi-8® 5% EC, Eminent® 12.5% EW, Abatchi® 25% and Punch® 40% EC reduced yellow rust disease severity to the lowest level

possible and revealed grain yield advantage better than the untreated application so that these fungicides included within management strategies. Furthermore, there is a need to continuously monitor the crop during the growing season so that maximum benefits can be achieved by applying the fungicides at appropriate time. In the end we would like to take attention about fungicides residues and their effect on human and wildlife.

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تقييم الكفاءة الابادية لبعض المبيدات الفطرية ضد مرض الصدا الاصفر في القمح وبعض التغيرات البيوكيماوية

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الملخص العربي

الصدا الاصفر في القمح يسببه فطر *P. striiformis* ويعتبر من الامراض المدمرة لمحصول القمح وهو منتشر في جميع مناطق زراعة القمح علي مستوي العالم مهددا انتاج القمح. يعتبر استخدام المبيدات الكيماوية لمقاومة امراض النبات من المكافحة المتكاملة والفعاله ولكن يجب ان يكون استخدامها أمن للانسان والبيئة. تم تقييم ثمانية مبيدات لمكافحة مرض الصدا الاصفر في القمح وهي مبيدات: تلت-فنج شو-اباتشي-كراون-سومي آت-باناش-ايمنت-مينار. تم تقدير متبقي هذه المركبات في كلا من الحبوب والتبن بعد الحصاد. وقد نفذت هذه التجريه في موقعين مختلفين خلال موسم القمح 2017/2018. اوضحت النتائج ان اقل شدة مرضية ١,٦٧ % سجلت لمركب تلت الذي اعطى كفاءة ابادية ٩٨,٠٧% و زيادة في ناتج الحبوب ووزن الـ ١٠٠٠ حبه بقيم ٢٩,٩٢ و ٧٣,٦١% على التوالي. يلية مركب كراون الذي اعطى نسبة ابادة للصدأ ٩٦,٩١% وايضا زيادة في ناتج الحبوب ووزن الـ ١٠٠٠ حبه بقيم ٢٤,٣٩ و ٦٩,٣٤% على التوالي. اوضحت نتائج تحليل البروتين والكربوهيدرات في عينات الحبوب والتبن وجود فروق معنوية داخل المعاملات. كما اوضحت نتائج تحليل متبقيات المبيدات في عينات الحبوب والتبن بعد الحصاد قيم ايجابية في كل العينات وكانت اعلى من النسب العالمية المسموح بها.

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