EFFICIENCY OF THYMOL AND CARVACROL IN ACTIVATION OF PATHOGENESIS RELATED GENES IN WHEAT AGAINST *PUCCINIA STRIIFORMIS*

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ABSTRACT

Genetic resistance, which can be succeeded by combining several resistance genes, is the most helpful technique for controlling stripe rust disease (*Puccinia striiformis*). Nineteen wheat commercial cultivars were assessed for stripe rust resistance and the existence of four resistance genes were validated. *Yr5* gene was found in all the studied genotypes in different sizes ranged from 467 bp to 581 bp, while *Yr18* appeared only in four studied genotypes. The potential of thymol and carvacrol as bioactive compounds was investigated against stripe rust disease. Using real-time PCR, the regulation of defense genes expression was assessed. The findings of the present investigation indicated that both thymol and carvacrol had protective effects against stripe rust. Treatments with thymol and carvacrol boosted the incubation and latent periods of the disease. Additionally, in both treatments, the level of infection was lower than the control group. A significant up-regulation of the defense genes in wheat (*PR5*, *PR6*, *PR8* and *PR12*) was recorded after treatment with thymol (100 mg/l) for 0, 1, 3, 4, 8 and 14 days after inoculation (DAI) and the fold change was close to the transcription of *PR* genes in the resistance monogenic line *Yr15*. After inoculation, carvacrol (100 mg/l) induced a significant up-regulation in the expression of *PR* genes at all days, except at 3 DAI (for *PR6* and *PR8*) and 4 DAI (for *PR6* and *PR12*). These results suggest that thymol and carvacrol may be utilized as elicitors of systemic resistance to stripe rust in wheat.

Keywords: Wheat; stripe rust; *Yr* Resistance genes; Thymol; Carvacrol; induced resistance; Pathogenesis related genes.

INTRODUCTION

Wheat is a widely cultivated crop around the globe, with 218 million hectares harvested in 2017 (FAO, 2017). One of the most critical variables affecting wheat yield is the occurrence of plant diseases. Wheat rust diseases have long been considered one of the most severe biotic production impediments worldwide. *Puccinia striiformis* f. sp. *tritici* (causes stripe rust), *P. graminis* f. sp. *tritici* (causes stem rust), and *P. triticina* (causes stripe rust) are three pathogens of wheat rust diseases. Stripe rust is a common disease throughout the winter and early spring (Chen, 2005; Ali *et al.,* 2017). It causes production losses ranging from 10% to 70% in most wheat-growing areas (Chen, 2020). Cultivating resistant cultivars, using the right fungicide, and following the right cultural practices may all help to manage the disease. For more effective, economical, and long-term management of stripe rust, more research is needed to establish additional cultivars with sustainable and high-level resistance to compete pathogen virulence.

The best approach for controlling stripe rust disease is the development of resistant cultivars, however, the traditional breeding technique is insufficient. A total of 43 resistance genes have been detected in wheat plants. There are a large number of genes that are race-specific, and wheat cultivars that contain some of these genes have considered an important tool in breeding programs (Cao *et al.,* 2001). Since molecular markers for particular stripe rust genes have been developed, detection of these genes can now be done regardless of phenotype. Molecular breeding based on marker assisted selection (MAS) may substantially improve breeding efficiency (Yu *et al.,* 2004). The availability of closed associated DNA markers is a requirement for using MAS in traditional breeding. This may increase the development of resistant genotypes and therefore, serves as an adequate method for plant breeding (Koebner *et al.,* 2003). To improve parental selection for crossover and monitoring genes in segregating progeny, markers may be employed to better define parental material (William *et al.,* 2007).

Thymol and carvacrol as natural plant volatile compounds showed high antifungal effects (Soković *et al.,* 2009; Spadaro and Gullino, 2014). These prospective fungicidal chemicals' mechanism of action has yet to be completely investigated. However, the phenolic structures of these compounds are very effective against pathogens. The performance of thymol and carvacrol is frequently due to the combination of various chemical compounds (Spadaro and Gullino, 2014; Banani *et al.,* 2018). However, nothing is known about PR gene induction's role of plant essential oils against plant pathogens.

Plants use a variety of immunological mechanisms, including pathogen detection, defense signal pathway activation, and the synthesis of antifungal substances such as PR proteins, all of which help to decrease pathogen invasion and reproduction (Sels *et al.,* 2008). PR proteins, for example, are good targets for developing long-lasting and diverse resistance to fungal pathogens (Ali *et al.,* 2017). Several transcriptional findings showed that *PR* genes are up-regulated in various crops following fungal infections, indicating that they play a role in disease resistance. *PR* gene expression is low under normal conditions, but it rises following fungal infection in both infected and non-infected parts of the plant, triggering resistance pathway (Návarová *et al.,* 2012; Esmail *et al.,* 2020; Salman *et al.,* 2021). Furthermore, numerous *in vivo* experiments have shown that PR proteins target or hydrolyze fungal cell walls, causing cell death. *PR5* and *PR12* have long been thought to be the most effective antifungal proteins in plants. Furthermore, overexpression of *PR* genes, either alone or in combination, improves resistance against fungal pathogens in different crops (Dai *et al.,* 2016). PR proteins are found across the plant kingdom and are made up of 17 different protein families. These proteins include thaumatin-like proteins (*PR5*), proteinase inhibitors (*PR6*), endochitinases (*PR8*) and defensins (*PR12*). Although these PR proteins are known to play key roles in plant resistance to different stresses, little is known about their involvement in wheat-*P. striiformis* interactions (Farrakh *et al.,* 2018).

This study aimed to validate the roles of DNA markers (*Yr5, Yr10, Yr15* and *YR18*) in stripe rust resistance in different wheat genotypes and to identify the effects of thymol and carvacrol as inducers of resistance against stripe rust through measuring the transcription levels of different *PR* genes.

MATERIALS AND METHODS

All the greenhouse experiments were performed in Agriculture Research Center, Giza, Egypt. Field experiments were conducted in two locations Sakha (6-27 ºC temperature and 36-55% relative humidity) and Gemmeiza (5-28 ºC temperature and 35-54% relative humidity).

Evaluation of the response of wheat varieties to stripe rust disease under field conditions: Wheat varieties containing 23 stripe rust resistance genes and another 19 varieties were tested for stripe rust resistance at adult plant stages. During the 2017/18 (8-27 ºC temperature and 36-54% relative humidity), 2018/19 (6-28 ºC temperature and 35-56% relative humidity), and 2019/2020 (5-26 ºC temperature and 36-55% relative humidity) growing seasons, adult plant resistance was assessed in the field on the same set of materials. During the season (at the booting stage), the required agricultural methods for inoculation were carried out according to Tervet and Cassell (1951). The modified Cobb's Scale was utilized to determine the severity of the disease (Peterson *et al.,* 1948). According to Roelfs *et al.* (1992), infection responses were classified as susceptible (S), moderately susceptible (MS), moderately resistant (MR) and resistant (R).

Genomic DNA extraction: The genomic DNA of wheat plants was obtained from fresh leaves (100-150 mg) following the CTAB (Cetyltrimethyl Ammonium Bromide) method (Murray and Thompson, 1980). The A260/A280 ratio was verified for measuring DNA concentration. For PCR amplification, high quality DNA samples were used. The stocks were diluted to a final concentration of 40 ng/l of DNA before being utilized in further experiments.

DNA marker analysis: Four DNA primers related to *Yr5, Yr10, Yr15* and *Yr18* resistance genes as shown in Table (1) were screened. Results of these SSR markers were considered for evaluating molecular differences among 19 wheat genotypes with *Yr5, Yr10, Yr15* and *Yr18* monogenic lines as positive controls.

All PCR products were run on 3 % agarose gel containing 5 µl of ethidium bromide (1 µg/ml). Along with the samples, a conventional DNA marker was run. The separated bands were photographed using a photo Doc-It^TM Imaging System and examined after being verified with a Benchtop UV-transilluminator.

Band scoring: Data for DNA markers were scored for computer analysis and the numbers and sizes of alleles produced by each primer were recorded and analyzed by GelAnalyzer3 program. DNA ladder marker 1 kb (+) (enzynomics) was used as the reference. Each polymorphic DNA fragment was treated as a separate locus since it corresponded to a distinct position in the genome.

Control of stripe rust disease by thymol and carvacrol under greenhouse conditions: Wheat (*Triticum aestivum* L.) seedlings of Egyptian genotype Gemmeiza-11 were put in a growth chamber at 20 ºC and subjected to a photoperiod of 12 h. Seedlings after seven days were transferred to a greenhouse, and infected with rust urediniospores. Depending on the infection types generated, seedling response was recorded as resistant (R) or susceptible (S) (Table 1). Seedlings were sprayed with

100 mg/l thymol (base chemicals India company) and carvacrol (Sigma-Aldrich Co.) at 24 h before and after inoculation. The period from inoculations to the onset of the first pustule is considered the incubation period (IP). The latent period (LP) was determined according to Parlevliet (1975) by adding up the number of pustules seen on marked leaves per day until no pustules appeared. In addition, the period from inoculation to the development of 50% of the pustules was reported. The size of 20 pustules were measured to compare pustule size on the first leaf. The number of pustules per unit leaf area (2.0 × 0.5 cm²) on the top surface of the leaves was calculated using the technique reported by Parlevliet and Kuiper (1977).

Table 1: List of four DNA marker sequences used in validation of resistance genes in wheat.

Resistance gene	Marker	Marker type	Primer Sequence (5′ → 3′)	Expected product size (bp)	Reference
Yr5	STS-7 STS-8	STS	GTACAATTCACCTAGAGT GCAAGTTTTCTCCCTATT	478	Chen *et al.* (2003)
Yr10	*Xpsp3000*	SSR	F: GCAGACCTGTGTCATTGGTC R: GATATAGTGGCAGCAGGATACG	260	Wang *et al.* (2002)
Yr15	*Xbarc8*	SSR	F: GCGGGAATCATGCATAGGAAAACAGAA R: GCGGGGGGCGAAACATACACATAAAAACA	190	Murphy *et al.* (2009)
Yr18	*Cssfr1*	SSR	F: TTGATGAAACCAGTTTTTTTTCTA R: GCCATTTAACATAATCATGATGGA	517	Lagudah *et al.* (2009)

RNA isolation and RT-qPCR procedures: Leaves were harvested at the following time points; 0, 1, 3, 4, 8 and 14 days after inoculation (DAI). These time intervals were chosen based on the identified stages of the infection process: [1] interaction between *P. striiformis* and plants (0 DAI, just inoculation), [2] *P. striiformis* entered plant tissue (1 DAI), [3] haustoria formation and starting of the life cycle (3 and 4 DAI), [4] disease symptom appearance (8 DAI) and [5] sporulation (14 DAI) (Farrakh *et al.*, 2018; Zhang *et al.*, 2012). For Gemmieza-11 genotype, control plants (inoculated only with *P. striiformis*) were subjected to the same procedure, using distilled water. Inoculated plants without thymol and carvacrol treatments of *Yr15* resistance monogenic line were used as a positive control and the non-inoculated plants of both genotypes (Gemmieza-11 and *Yr15*) at all different time points were used to normalize folding changes of each gene. The leaves were wrapped in labeled aluminum foil and cleaned with 95% ethanol before being frozen in liquid nitrogen and kept at –80°C until RNA isolation.

RNA extraction and cDNA synthesis: Total RNA was purified from wheat samples using Trizol reagent (iNtRON Biotechnology, Inc.) as directed by the manufacturer. Electrophoresis on a 2% ethidium bromide stained agarose gel was used to confirm the integrity of the RNA. The Topscript™ CDNA synthesis kit (enzynomics, Korea) was used to synthesize cDNA from a concentration of 2 µg RNA (Elsharkawy *et al.*, 2012).

qRT-PCR assay: For qRT-PCR analysis, four specific primers were used to determine the expression of pathogenesis related genes *PR5*, *PR6*, *PR8* and *PR12*, with the *β-tubulin* as a housekeeping gene. Primers were used from previous studies as presented in Table (2). Ten microliters (µL) of TOPreal™ qPCR 2X PreMIX (SYBR green with low ROX, enzynomics, Korea), 0.5 µM of each primer, and 2 µL of cDNA were used in the reaction. Detection of gene expression was done in triplets using the Thermocycler (Bio-Rad, *C-1000*) real-time PCR system. The program was set up in the first of an initial denaturation at 94 °C for 5 min followed by 40 cycles comprising of: 94 °C for 30 s, annealing at 55 °C for 30 s and 72 °C for 1 min. The relative expressions were calculated by the $2^{-\Delta\Delta ct}$ method described by Livak and Schmittgen (2001) and values of each gene have normalized its folding changes against the housekeeping gene (*β-tubulin*) and the control values (non-inoculated plants) at each time point.

Statistical analysis: The experiments were conducted using a completely randomized approach and one-way ANOVA was used. Three repetitions were conducted of all experiments. Fisher's least significant difference test was used to statistically analyze the data. Analysis was done by XLSTAT Pro statistical analysis software (Addinsoft, New York, USA) at $P \leq 0.05$.

Table 2: Accession numbers, function and annealing temperatures of PR genes used for qRT-PCR assay.

PR-Proteins	Accession No.	Function	Annealing temperature	Reference
PR5	NW_015793016	Thaumatin-like proteins	52 °C	Van Loon (1982)
PR6	NW_004196001.1	Proteinase inhibitor	56 °C	Green and Ryan (1972)
PR8	NC_026660.1	Class III Chitinase	60 °C	Metraux *et al.* (1988)

| PR12 | NC_025209.1 | Defensin | 58 °C | Terras *et al.* (1995) |
| β-tubulin | U76897 | Reference gene | 60 °C | Scholtz and Visser (2013) |

RESULTS AND DISCUSSION

Efficiency of *Yr* genes against stripe rust disease in adult plants: Under natural infection, the efficiency of resistance genes (Yr's) in the adult plant stage was evaluated. During three seasons 2017/18, 2018/19, and 2019/20, the genotypes were tested at two sites, Sakha and El-Gemmeiza. During the first growing season, the monogenic lines *Yr1, Yr5, Yr10, Yr15, Yr17, Yr27, Yr CV* and *Yr SP* grown in Sakha and El- Gemmeiza were resistant to stripe rust. Meanwhile, *Yr1, Yr5, Yr10, Yr15, Yr17, Yr27, Yr CV* and *Yr SP* were resistant to stripe rust in the second growing season. Lastly, the lines *Yr1, Yr5, Yr10* and *Yr15* were resistant to stripe rust in the third growing season (Table 3).

The evaluation of stripe rust resistance in the field indicated that *Yr5*, *Yr10*, *Yr15* and Sakha 61 were resistant genotypes in the three studied seasons at different locations Sakha and Gemmeiza. However, this study indicates that the resistance genes *Yr5*, *Yr10*, *Yr15* and *Yr18* and other *Yr* genes could contribute to resistance for stripe rust pathogen. The obtained results are in accordance with those reported by Sadravi (2008); Pourkhorshid *et al.* (2014); Zhou *et al.* (2014). Since *Yr5* is a race-specific resistance gene, it should be combined with other potent genes and/or race- non-specific adult-plant resistance genes. A combination like this may offer excellent resistance against stripe rust (Yan *et al.,* 2003).

Field assessment of Egyptian wheat cultivars: The varieties Sakha 61, Sakha 94, Sakha 95, Giza 168, Giza 171, Gemmeiza 12, Sids 14 and Misr 3 were resistant to stripe rust disease in the first growing season (Table 4).

Meanwhile, Sakha 61, Sakha 95, Giza 171, Sakha 94, Misr 3 and Gemmeiza 12 were resistant in the second growing season and Sakha 93, Sakha 8, Sakha 69, Sids 11, Sids 13, Sids 14, Gemmeiza 7, Gemmeiza 9, Misr 1 and Misr 2 were resistant in the third growing season (Table 4).

The collected data suggested that the severity of stripe rust was lower in the growing season 2017/18 than in 2018/19 and 2019/20 growing seasons. The rust data also revealed that the severity of disease at Sakha was significantly higher than at El- Gemmeiza. Major or minor genes, or both, control disease resistance; nevertheless, a complimentary impact between major genes may increase the variety's response and result in higher degrees of resistance. Variable variations have diverse reactions depending on the genetic composition of the major genes (Simons *et al*., 1978; Omara *et al.,* 2021).

Validation of stripe rust resistance genes: The molecular analysis of 19 Egyptian wheat genotypes was performed using four stripe rust resistance genes selected based on their tight linkages at adult stage. For *Yr5* resistance gene, the results indicated that monogenic line *Yr5* separated a DNA fragment of 478 bp as a positive control (Figure 1), while it separated four alleles in the studied genotypes ranged from 467 to 581 bp with 114 bp size differences. Ten out of the 19 studied genotypes amplified a fragment of 478 bp as present in positive control indicating the presence of *Yr5* in these genotypes as shown in Table 3. The evaluation of stripe rust resistance in the field indicated that *Yr5*, *Yr10*, *Yr15* and Sakha 61 are resistant genotypes in the three studied seasons at different locations (Sakha and El-Gemmeiza).

Table 3: Response of 23 monogenic lines against stripe rust disease under field conditions at Sakha and El-Gemmeiza locations.

No.	Monogenic Lines	Rust reaction					
		Sakha			Gemmeiza		
		2017/18	2018/19	2019/20	2017/18	2018/19	2019/20
1	Yr 1	0	0	0	0	0	0
2	Yr2	20S	40S	30S	20S	50S	40S
3	Yr3	30S	50S	40S	30S	40S	50S
4	Yr4	40S	60S	50S	30S	50S	40S
5	Yr5	0	0	0	0	0	0
6	Yr6	80S	100S	90S	70S	100S	90S
7	Yr7	80S	100S	90S	90S	100S	80S
8	Yr8	70S	90S	80S	70S	80S	70S
9	Yr9	80S	100S	70S	70S	100S	80S
10	Yr10	0	0	0	0	0	0
11	Yr15	0	0	0	0	0	0
12	Yr17	0	40S	30S	10S	30S	20S
13	Yr18	70S	90S	100S	60S	80S	80S

14	Yr21	50S	60S	50S	40S	70S	60S
15	Yr25	40S	70S	60S	30S	70S	60S
16	Yr27	0	50S	30S	0	40S	30S
17	Yr28	20S	60S	50S	10S	50S	40S
18	Yr29	20S	60S	40S	30S	50S	50S
19	Yr CV	5R	20S	30S	5R	20S	10S
20	Yr SP	10R	20S	20S	5R	30S	10S
21	Yr SU	30S	50S	40S	20S	40S	20S
22	Yr SD	40S	60S	50S	30S	50S	40S
23	Yr A	80S	100S	90S	70S	100S	100S
	L.S.D 5%	12.360	10.182	8.401	9.700	8.401	8.010

Table 4: Response of 19 wheat varieties for strip rust under field conditions in 2017/18, 2018/19 and 2019/2020 growing seasons.

| No	Wheat variety	Location /Rust reaction					
		2017/18		2018/19		2019/20	
		Sakha	Gemmeiza	Sakha	Gemmeiza	Sakha	Gemmeiza
1	Sakha 8	90S	80S	90S	90S	80S	80S
2	Sakha 61	0	0	0	0	0	0
3	Sakha 69	80 S	70 S	90 S	90 S	80 S	70 S
4	Sakha 93	40S	30S	70S	80S	60S	50S
5	Sakha 94	5S	5S	10S	20S	5S	5S
6	Sakha 95	0	0	TrMS	TrMS	TrMS	TrMS
7	Gemmeiza 7	40S	50S	80S	90S	70S	70S
8	Gemmeiza 9	40S	50S	70S	80S	60S	50S
9	Gemmeiza 11	80S	80S	100S	100S	90S	80S
10	Gemmeiza 12	0	0	TrMS	TrMS	TrMS	TrMS
11	Giza 168	10S	10S	40S	50S	30S	40S
12	Giza 171	0	0	10S	20S	5S	5S
13	Sids 12	80S	90S	100S	100S	90S	100S
14	Sids 13	80S	70S	80S	90S	70S	50S
15	Sids 14	0	0	40S	50S	30S	20S
16	Shandaweil 1	0	0	50S	40S	40S	50S
17	Misr 1	10S	20S	80S	70S	80S	90S
18	Misr 2	10S	20S	90S	90S	90S	80S
19	Misr 3	0	0	TrMS	TrMS	TrMS	TrMS
	L.S.D 5%	12.120	8.582	11.819	9.645	11.328	10.819
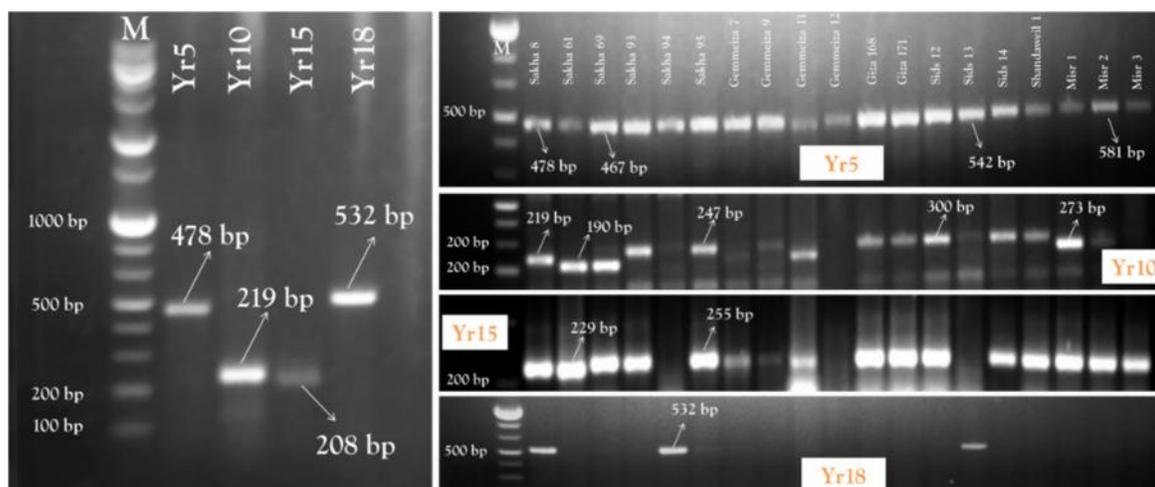
Figure 1. Band patterns of stripe rust resistance genes in 19 tested genotypes in addition to monogenic lines. M; 1 kb (+) DNA ladder.

As shown in Figure (1), stripe rust resistance dominant gene *Yr10* was validated in 19 wheat genotypes, the monogenic line *Yr10* (a band with size of 219 bp in the positive control) and two commercial varieties (Sakha 8 and Gemmeiza 7). Four alleles ranged in their molecular size from 190 to 300 bp were found in the rest genotypes.

For *Yr15* resistance gene, the corresponding allele found in the positive control *Yr15* monogenic line was 208 bp in size, while the 19 tested genotypes were divided into three groups. The first group containing the two genotypes, Sakha 8 and Sakha 61, presented an allele with molecular size of 229 bp, the second group (presented a 255 bp allele) appeared in 14 genotypes, while the rest three genotypes did not record any alleles for this gene.

In validation of *Yr18* stripe rust resistance gene, only four genotypes (Sakha-8, Sakha-94, Sakh-95 and Sids-13) out of the 19 studied were found to carry this gene and presented the same allele appeared in monogenic line *Yr18*. Therefore, it is likely that if the genotype does not carry the corresponding alleles presented in the positive control, the genotypes' resistance could be due to the presence of another effective gene. On the other hand, susceptibility of the genotype that has the corresponding alleles of resistance gene indicates that this gene cannot be expressed in this genotype.

New progress in the molecular analysis of resistance genes have allowed the development of direct markers that integrate significant race-specific resistance with adult plant resistance genes (Sharma, 2012). Bariana *et al.* (2002) reported that the varieties with the *Yr10* gene amplified a 258-260 bp fragment, but those without this gene only produce a 240 bp band fragment. This gene also expresses at the end of plant development, making it unsuitable for early selection of resistance to stripe rust. Additionally, this gene was present in the majority of cultivars and showed its effectiveness against the predominant and virulent pathotype 78S84 (Lagudah *et al.,* 2009; Mukhtar *et al.,* 2015). When combined with other resistance genes, this gene may improve protection against stripe rust (German and Kolmer, 1992). Based on previous studies, *Yr5* and *Yr10* genes were effective with high levels of resistance against all virulent races in North America (Bux *et al.,* 2012), Iran (Afshari, 2008) and China (Chen, 2005). This fact indicated that *Yr5* was a useful candidate for breeding programs.

Table 5: Absence/presence of corresponding alleles for four resistance genes in addition to other alleles in 19 Egyptian commercial wheat cultivars.

Number	Genotype	*Yr* resistance genes			
		Yr5 (478 bp)	*Yr10* (219 bp)	*Yr15* (208 bp)	*Yr18* (532 bp)
1	Sakha-8	+	+	229	+
2	Sakha-61	+	190	229	-
3	Sakha-69	467	190	255	-
4	Sakha-93	467	247	255	-
5	Sakha-94	+	247	-	+
6	Sakha-95	+	247	255	+
7	Gemmeiza-7	+	+	255	-
8	Gemmeiza-9	+	273	255	-
9	Gemmeiza -11	+	247	255	-
10	Gemmeiza-12	+	-	-	-
11	Giza-168	+	300	255	-
12	Giza-171	+	300	255	-
13	Sids-12	542	300	255	-
14	Sids-13	542	300	-	+
15	Sids-14	581	300	255	-
16	Shandaweil-1	581	300	255	-
17	Misr-1	581	273	255	-
18	Misr-2	581	300	255	-
19	Misr-3	581	300	255	-
	Total No. of alleles	4	5	2	1
	Polymorphism %	100	100	100	100
	Size range (bp)	114	110	26	0

+ indicates the presence of corresponding genes, - indicates the absence of genes

Effect of thymol and carvacrol on incubation, latent periods, and infection type: Figures 2 and 3 demonstrate the impacts of the thymol and carvacrol on stripe rust development, including incubation and latent periods and infection type. All parameters showed significant variations when different treatments were sprayed at 24 h before and after inoculation. Thymol and carvacrol treatments enhanced incubation and latent periods. Furthermore, as compared to the control, both treatments had the lowest infection type. However, the incubation and latent durations, as well as the infection type, grew more slowly than in the control treatment (Figs. 2 and 3).
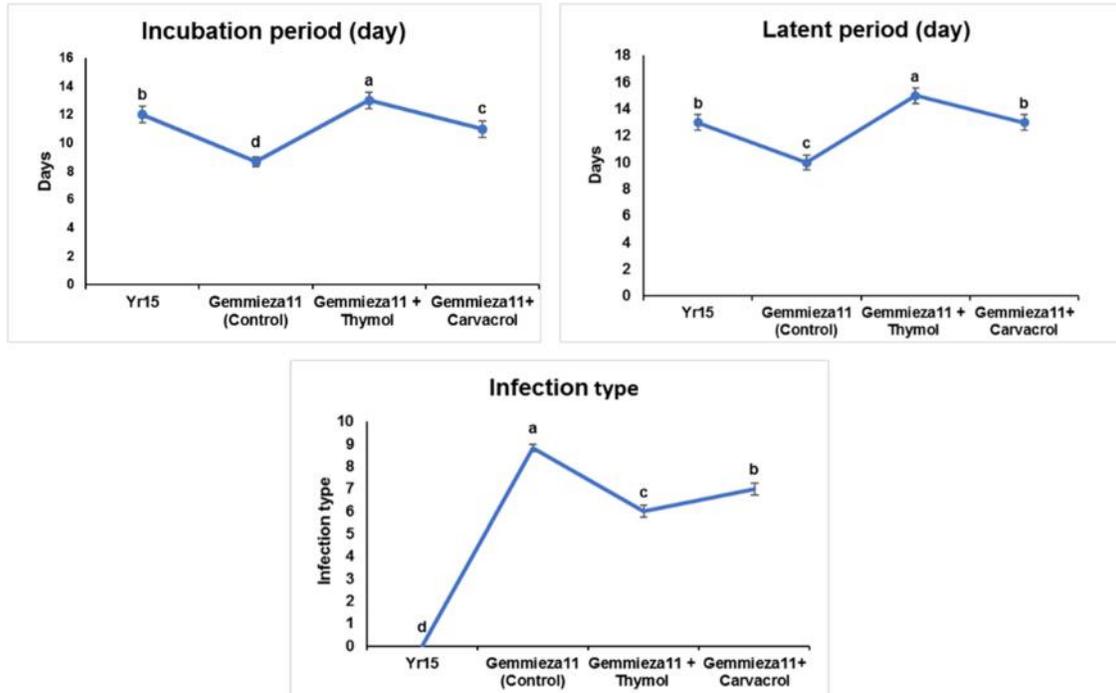

Figure 2. Effect of thymol and carvacrol on stripe rust development (incubation, latent periods, and infection type) at seedling stage.
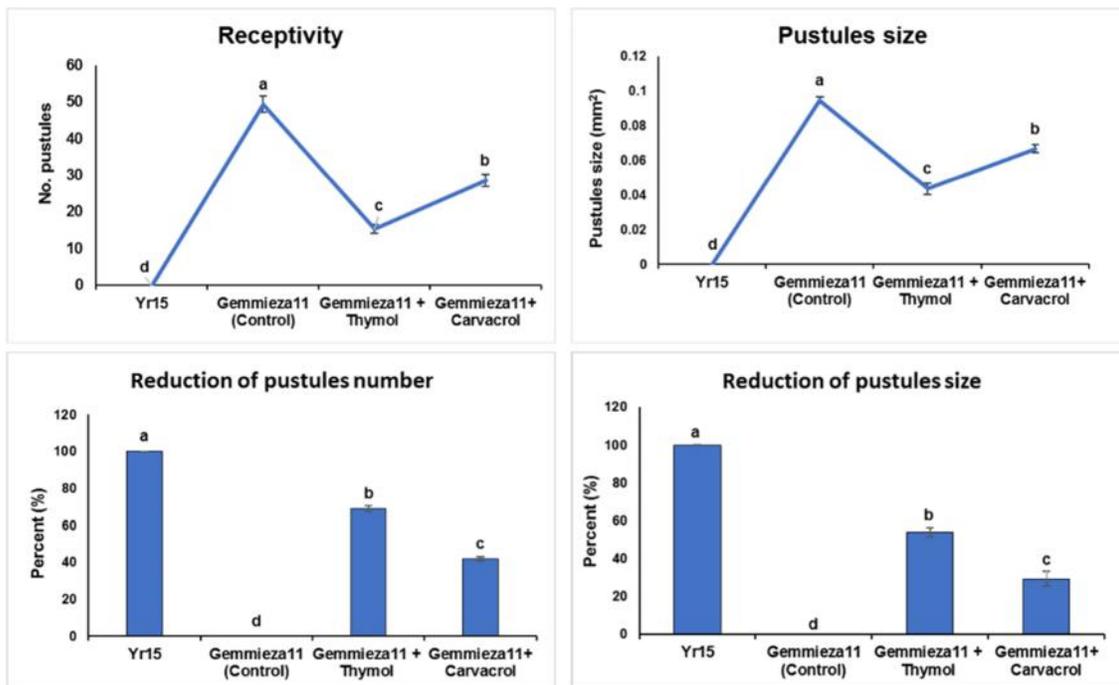
Figure 3. Effect of treatments on pustules size, number and receptivity of stripe rust urediniospores at seedling stage.

Plant extracts substantially extended the incubation and latent periods of wheat leaf rust (Omara *et al.,* 2015 and 2019). Since thymol and carvacrol are currently extensively used as feed additives, these compounds may be useful candidates in the control of *P. striiformis* in wheat. Several researchers examined the function of thymol and carvacrol against bacterial infection through down-regulating the virulence factor production (Inamuco *et al.,* 2011; Burt *et al.,* 2016). Such molecules may have direct antifungal activity that can prevent *P. striiformis* growth via inactivation of virulence genes but this needs further studies.

Effect of thymol and carvacrol on *PR* protein genes expression: In order to analyze the up-regulation of *PR* genes involved in thymol and carvacrol- stimulated resistance in wheat, the expression of *PR5*, *PR6*, *PR8* and *PR12* was analyzed in treated wheat plants at 0, 1, 3, 4, 8 and 14 days after inoculation (Tables 6, 7, 8 and 9).

All of the pathogenesis related genes that were examined showed up-regulation by thymol and carvacrol at different time points compared with non-treated plants (Figure 4). Treatment with 100 mg/l thymol in susceptible Gemmiza 11 genotype induced up-regulation of the expression of all tested *PR* genes at 0, 1, 3, 4, 8 and 14 days after pathogen inoculation (DAI) compared to inoculated control plants. The fold change was very close to the expression of *PR* genes in the resistance monogenic line *Yr15*. While, carvacrol treatment (100 mg/l) showed significant differences of relative expression of *PR* genes at all days of inoculation compared to control, except after 3 DAI in *PR6* and *PR8* and at 4 DAI in *PR6* and *PR12*. Also, treatment with carvacrol recorded down-regulation of *PR8* at 14 DAI and *PR12* at 1 and 3 DAI. These results suggest that thymol treatment was better than carvacrol in inducing resistance against *P. striiformis* in wheat.
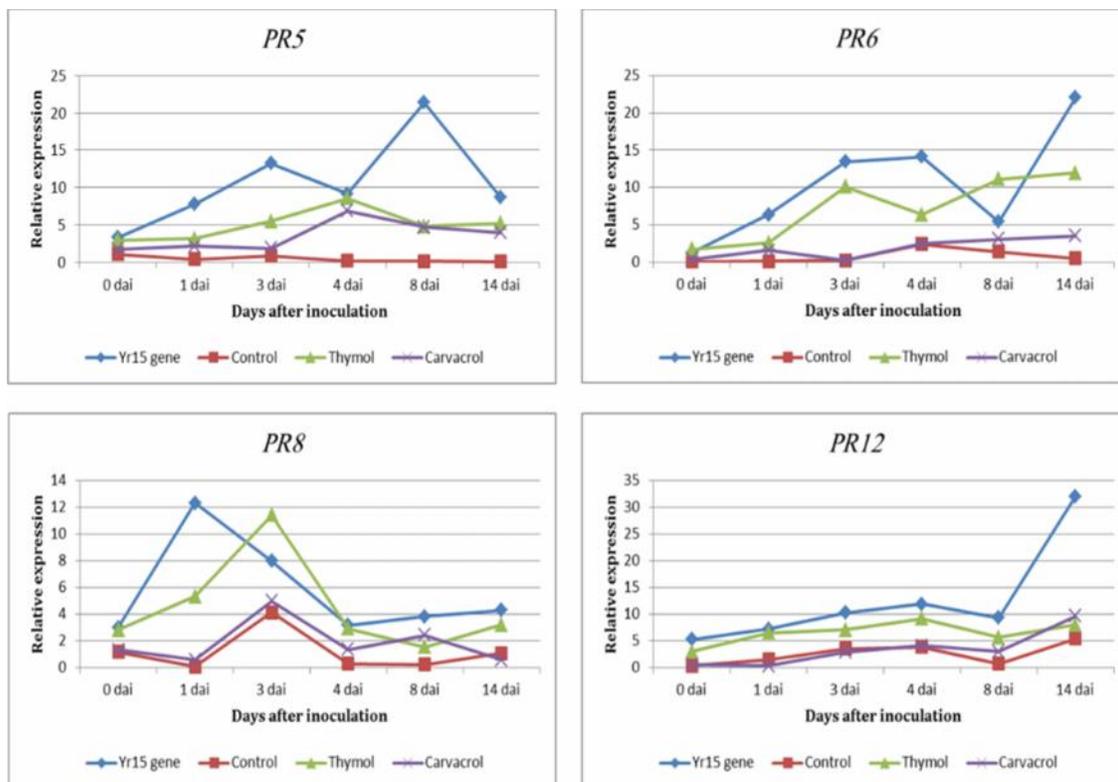

Figure 4. Expression of pathogenesis-related protein genes *PR5*, *PR6*, *PR8* and *PR12* in wheat treated with thymol and carvacrol at different days after inoculation.

Data from this study supported the effect of thymol and carvacrol in regulation of gene expression. In wheat, thymol and carvacrol have properties of inducers that can activate the resistance genes. Thyme oil enhanced apple resistance to *B. cinerea* because it encouraged the expression of *PR8*. Although this signal stimulated the

plant, thyme produced a modest induction of resistance (*PR8* expression level was 2.5-fold greater than the expression in water-treated fruits) after 6 h of pathogen inoculation (Banani *et al.,* 2018). Also, Giovagnoni *et al.* (2020) confirmed that thymol and carvacrol concentrations of 0.47 mM inhibited cell invasion by

lowering the expression of virulence genes such as *SipA* and they indicated that treatment with carvacrol showed expression significantly lower than thymol in all studied genes, except *SipC.*

Table 6: Relative expression of pathogen-related protein gene *PR5* in Gemmieza-11 wheat genotype at different days after inoculation (DAI) with *P. striiformis* at seedling stage in comparison with *Yr15* resistance monogenic line.

Days after inoculation	*Yr15* gene	Gemmieza-11		
		Control	Thymol	Carvacrol
0	3.34	1.07[c]	2.96[a]	1.73[b]
1	7.79	0.42[c]	3.14[a]	2.12[b]
3	13.25	0.84[c]	5.48[a]	1.85[b]
4	9.18	0.21[c]	8.57[a]	6.89[b]
8	21.38	0.16[b]	4.81[a]	4.72[a]
14	8.65	0.08[c]	5.22[a]	3.99[b]

Table 7: Relative expression of pathogen-related protein gene *PR6* in Gemmieza-11 wheat genotype at different days after inoculation (DAI) with *P. striiformis* at seedling stage in comparison with *Yr15* resistance monogenic line.

Days after inoculation	*Yr15* gene	Gemmieza-11		
		Control	Thymol	Carvacrol
0	1.26	0.06[c]	1.76[a]	0.44[b]
1	6.39	0.12[c]	2.59[a]	1.60[b]
3	13.46	0.27[b]	10.13[a]	0.25[b]
4	14.12	2.43[b]	6.38[a]	2.49[b]
8	5.45	1.40[c]	11.14[a]	3.09[b]
14	22.06	0.54[c]	11.98[a]	3.52[b]

Table 8: Relative expression of pathogen-related protein gene *PR8* in Gemmieza-11 wheat genotype at different days after inoculation (DAI) with *P. striiformis* at seedling stage in comparison with *Yr15* resistance monogenic line.

Days after inoculation	*Yr15* gene	Gemmieza-11		
		Control	Thymol	Carvacrol
0	3.00	1.16[b]	2.82[a]	1.34[b]
1	12.31	0.09[c]	5.33[a]	0.56[b]
3	7.97	4.13[b]	11.41[a]	4.99[b]
4	3.16	0.29[c]	2.91[a]	1.35[b]
8	3.83	0.24[c]	1.53[b]	2.42[a]
14	4.29	1.07[b]	3.19[a]	0.57[c]

Table 9: Relative expression of pathogen-related protein gene *PR12* in Gemmieza-11 wheat genotype at different days after inoculation (DAI) with *P. striiformis* at seedling stage in comparison with *Yr15* resistance monogenic line.

Days after inoculation	*Yr15* gene	Gemmieza-11		
		Control	Thymol	Carvacrol
0	5.26	0.31[c]	3.01[a]	0.47[b]
1	7.18	1.53[b]	6.51[a]	0.38[c]
3	10.24	3.56[b]	7.08[a]	2.94[c]
4	11.89	3.89[b]	9.17[a]	4.11[b]
8	9.31	0.72[c]	5.66[a]	3.02[b]
14	32.04	5.40[c]	7.99[b]	9.65[a]

Conclusion: Stripe rust resistance-related genes were investigated in 19 commercial wheat cultivars using four DNA markers in this research. Genetic differences of various genes were observed in the genotypes evaluated. Stripe rust resistance in wheat genotypes may be improved by incorporating favorable alleles from this study. *P. striiformis* resistance in wheat was achieved using 100 mg/l thymol and carvacrol. According to gene expression data, low-molecular weight proteins, such as thymol and carvacrol, have a role in the signaling pathway of the resistance in the early stages of stripe rust disease via the interaction of multiple *PR* genes.

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