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CHEMICAL INDUCTION OF SYSTEMIC AQUARID RESISTANCE AND EFFECT OF THEIR APPLICATION TIME ON RICE BLAST "*MAGNAPORTHE GRISEA*" INFECTION TO CULTIVARS SAKHA101 AND SAKHA104.

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ABSTRACT

Blast is the most destructive rice diseases in Egypt and could cause significant yield losses. The variable nature and race shifting of the pathogen often leads to resistance breakdown of high yielding varieties such as Sakha 101 and Sakha 104. Chemical control is not always effective and often has an undesirable impact on the environment and human health. Some antioxidants and organic compounds; Bion (BTH, benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester), at concentrations of 0.1, 0.3 and 1 mM; Salicylic acid 8 mM (SA), Benzoic acid 8 mM, Nicotinic acid 8mM, H2O2 30%, and Compost tea 100% were directly applied as foliar spray of 21-days old seedlings prior to challenge inoculation with *Magnaporthe grisea* to promote blast resistance in rice leaves. Under greenhouse condition, artificial inoculation was applied after four fixed periods (5, 10, 15 and 20 days after treatment (DAT)) from the application date of all antioxidants. All antioxidants and compost tea reduced the infection percentage compared with untreated check especially with the inoculation 5 DAT. Concerning infection % of rice blast, there are a remarkable significant differences among all antioxidants compared with control. All antioxidants were significantly reduced the infection percentage. Salicylic acid was the most effective antioxidant at concentration of 8 mM, it recorded 4.9, 9.7, 28 and 15.5 % with 5, 10, 15 and 20 DAT, respectively. Also, Bion (BTH) at both concentrations 0.3 and 1.0 mM exhibited a significant reduction in infection %. The rest of antioxidants exhibited significant increase in infection % especially under late induced periods from 10-20 DAT. The optimal period of induced resistance, was artificial inoculation 5 DAT which recorded the lowest infection percentage compared with the inoculation after 10 to 20 days. With both Salicylic acid and Bion, artificial inoculation 5 DAT to 15 days led to a significant reduction in disease infection percentage and severity. While the resistance was remarkably sharply decreased after 15 days from induced resistance treatment with all antioxidants. Optimal induced period of Benzoic acid, Nicotinic acid, H2O2, Compost tea and Cinnamic acid was continued from 5 to 10 days only. Treatment with salicylic acid at 8 mM recorded the lowest area under disease progress curve (AUDPC), 409.48 compared by the control 1304.24, followed by Bion at 0.3 and 1.0. However, BTH and Salicylic acid had neither suppression nor fungicidal effect on linear growth, spore germination, sporulation and appressorium formation of *Magnaporthe grisea* in vitro. Concerning Peroxidase (PO) activity with both SA 4mM and BTH increased continuously in 0-25 min. intervals and markedly significant increase, although PO activities reach to be maximum at 25 min. in which was higher than that control and other treatments. SA had the highest activities in period intervals followed by BTH. Compost Tea and H_2O_2 showed no significant increase in PO activity with all time intervals while the other treatments showed PO activities only in 10-20 min. compared to control. Polyphenoloxidase (PPO) activity has gradually decreased during intervals 0 to3 min. Spraying by SA 4mM recorded the highest enzyme activity in all intervals and markedly significant increase in activity. For growth characters, all applications of antioxidants treatments led to a significant increase in leaf area, plant height, chlorophyll content, fresh and dry weight of rice leaves compared to control. In general, Sakha 101 rice cultivar exhibited the highest response to applications of all antioxidant compared with other cultivar Sakha 104 that have a weak response of growth characters to antioxidants application.

Keywords: Rice blast, *Magnaporthe grisea*, induce resistance, salicylic acid, BTH, systemic acquired resistance

Abbreviations: BTH, benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester; JA, jasmonic acid; SA, salicylic acid; SAR, systemic acquired resistance.

INTRODUCTION

Rice is the second important cereal crop in the world. It is essential for 54% of the world's population. In Egypt, rice is the second staple food after wheat. It is annually grown in more than one million feddan (420,000 ha), mostly in the Northern part of the Nile delta, which produces about 6 million tons of paddy rice/year, with an average of about 10.06 tons/hectare (RRTC, 2009). In the near future, this quantity will not be sufficient because of the dramatic increase in local population and constraints due to many abiotic and biotic factors.

Blast disease is considered a major constraint for maximum yield production because of its high level of variability and quick spread in case of susceptible cultivars. Blast is the most destructive rice disease in Egypt and could cause significant yield losses. The variable nature and race shifting of the pathogen often leads to resistance breakdown of high yielding varieties such as Sakha 101 and Sakha 104 (Couch and Kohn 2002 and Sehly *et al*. (2008).

Traditional methods used to protect crops from diseases have been largely based on the use of chemical pesticides. Excessive and improper applications of fungicides can have drastic effects on the health of humans, animals and environment. Chemical methods with repeated use are not economical in the long run because they pollute the atmosphere, damage the environment, leave harmful residues and lead to the development of resistant strains among the target organisms (Naseby *et al*., 2000). Many of these chemicals are also too expensive for the resource of poor farmers. A reduction or elimination of synthetic pesticide applications in agriculture is highly desirable. One of the most promising means to achieve this goal is by the use of new tools based on biocontrol agents for disease control alone or integrated with reduced doses of integrated chemicals in the control of plant pathogens resulting in minimal impact of the chemicals on the environment (Harman and Kubicek, 1998). Therefore, new approaches are being explored to suppress diseases through eco-friendly means as systemic acquired resistance inducers. For many years, it has been realized that the defense of a plant is not only restricted to the pathogen-attacked tissues, but also extends to distal tissues which become more resistant to a second challenge by the same or another pathogen. Ross (1961) termed this phenomenon 'systemic acquired resistance' (SAR). In subsequent years, SAR was observed in a number of plants and was effective against a broad range of pathogens (Kuc, 1982 and Ryals *et al.,* 1996). While many studies have focused on SAR model plants such as cucumber, tobacco or Arabidopsis, the existence of SAR in monocotyledonous plants, especially cereals, is still a matter of debate, and the reports on a SAR-like phenomenon in cereals are scarce (Smith and Metraux, 1991).

Plants have evolved methods to protect themselves with a unique self-protection system as well as various morphological adaptations. The primary response in this self-protection system involves specific pathogen recognition and a rapid induction of localized host cell death, (Ross 1961). The secondary response consists of induced resistance to protect the plant's body from further pathogenic attacks ,Kuc 1982. These responses are governed by hormonal regulation, in which salicylic acid (SA), jasmonic acid (JA) and ethylene can each contribute. Plants can also activate distinct defense signaling pathways, depending on the type of invading pathogen (Maleck and Dietrich 1999, Pieterse and Van Loon 1999). SAR is activated after infection by a necrotizing pathogen and confers resistance against a broad spectrum of pathogens in uninfected parts of the plant (Durner *et al*. 1997). SAR is an inducible defense mechanism that plays a central role in disease resistance (Hammerschmidt and Kuch 1995). Certain safety chemicals such as phosphate salts, isonicotinic acid, jasmonic acid, salicylic acid (SA), 2,6-dichloroisonicotonic acid (INA), and b-aminobutyric acid, can induce SAR in many pathogen-plant system (Hammerschmidt and Kuch 1995, Siegrist *et al.* 1997). SA has been proposed as the systemic signal for the induction of SAR. It has been suggested that SA is synthesized at the site of pathogen-induced necrosis and is translocated to induce SAR in uninfected leaves. Recent studies have shown the effectiveness of benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) as a novel crop protecting agent which does not itself have anti-microbial properties, but instead increases crop resistance to disease by activating the SAR signal transductioin pathway (Gorlach *et al*., 1996 and Cole 1999).BTH was able to prime key defense genes such as phenylalanineammonia lyase (PAL); PR proteins viz., chitinase (CHI)m 1,3 glucanase (GLU) and peroxidase (POD) as well as signal transduction pathway genes in cucumber against anthracnose disease (Bovie *et al*., 2004) and activates oxidative burst enzymes in pear against scab (Faize *et al*., 2004). Although BTH is highly effective as inducing enhanced disease resistance, its mode of action and cellular targets are unclear. Nevertheless, rice in the field is protected against *Magnaporthe grisea*, the causal agent of the rice blast disease by Probenazole (3- Allyloxy-1,2-benzis-othiazol-1,1-dioxid) that is not fungicidal but probably possesses plant immunizing activity (Sekizawa and Mase, 1981). In other words, the concept of acquired resistance seems to be applicable to rice in the field. Another agrochemical, 2,6-dichloroisonicotinic acid (INA) was found to improve the resistance of rice against *M. grisea* and *Xanthomonas oryzae* (Smith and Metraux, 1991). Both compounds seem to transform susceptible rice cultivars into resistant cultivars and the plant reactions to *M. grisea* of these conditioned, formerly susceptible, plants are similar to genetically resistant cultivars (Seguchi *et al.*, 1992; Sekizawa and Mase, 1981). Certain chemicals such as salicylic acid (SA) and benzothiadiazole derivatives (BTH) can enhance resistance to subsequent attack, not only at the site of treatment, but also in tissues distant from the initial treatment sites. Recently, they have been developed as a potent systemic acquired resistance activation signal transduction pathway in several plant species, Bokshi *et al*. (2003) and Achou *et al*. (2004). Narusaka *et al*. (2001) and Bovie et al. (2004) added that BTH is able to prime key defense genes such as PR proteins vis, chitinase ,1,3 gluconase and peroxidase as well as signal transduction pathway genes in cucumber against anthracnose disease . In rice, BTH treatment of seedlings gave season long protection against rice blast infection ,as well as BTH and SA induce disease resistance in plants , Gorlach *et al*.(1996) . Padmaja *et al*. (2004) Chakraborty *et al.* (2005) added that elicitation of defense enzymes (chitinase, gluconase and peroxidase) were done with the treated plants by SA showing resistant reaction against blister blight disease. Furthermore, Morris *et al.* (1998), Malolepsza (2005) and Phuntumart *et al.* (2006) added that pretreatment of maize with SA, tomato with BTH and cucumber with SA, BTH reduced leaf spot, gray mold and anthracnose infection development, respectively.

Compost extracts contain biocontrol agents, as well as unidentified chemical factors that appear to play a role in efficacy (Weltzien, 1992 and Cronin *et al*. 1996). Although the mechanisms by which these extracts provide control remain largely a mystery, (Cronin *et al*. 1996) demonstrated that a low molecular weight compound was critical for in vitro lysis of conidia of *Venturia inaequalis*. It has been postulated that the protective effects of compost extracts is due, at least in part, to the induction of systemic resistance in plants Weltzien, 1992. Zhang *et al.* (1996) reported that peroxidase activity, a putative SAR marker in cucumber (Graham and Graham, 1991 and Rasmussen *et al.*, 1995), was significantly higher in plants grown in a compost mix than in plants grown in a peat mix. They concluded that the interaction of the compost and pathogen infection appeared critical for rapid activation of SAR-associated gene expression in cucumber plants grown in compost mix, Zhang *et al*. (1996).

In this paper, we outline our interest in two aspects of acquired resistance of rice blast fungus *M. grisea*. Firstly, we are interested in the role and expression of some chemical inducers especially SA and BTH in mediating and build-up of acquired resistance prior to challenge inoculation with *M. grisea* and plant defense reactions after challenge by the pathogen. Secondly, we are interested in determining the optimal time conditioning of rice in response to exogenous application of chemical inducers.

MATERIALS AND METHODS

Plant Materials and Growth Conditions:

During 2010 season, grains of both rice cultivars Sakha101 and Sakha 104 were seeded in plastic trays (30 x 20 x15 cm). Each tray had 6 rows, each row representing one treatment. Seedlings were ready for treatment at 3-leaf stage, about 3-weeks after sowing. The second expanded lower leaf of young seedlings was treated with each treatment prior to blast fungus inoculation to induce the systemic protection and movement of chemical inducer from lower leaf to the upper ones.

Chemical Inducer Treatments:

To induce resistance, fresh solution of BTH , 50% wettable granule formulation , Bion, Novartis, Basel, Switzerland) at concentrations of 0.1, 0.3 and 1 mM;, Benzoic acid 8 mM, Nicotinic acid 8mM, H2O2 30%, Compost tea 100% were directly dissolved in deionized water plus 0.02 % Tween 20.

Both Salicylic acid 8 mM (SA) and Cinamic acid 8mM were first dissolved in few drops of absolute ethanol then add water plus Tween. All rice plants were applied with all inducers when the plants had three expanded leaves. Rice plants were treated with a chemical inducer by hands. Control plants were sprayed evenly with a 0.02% (v/v) Tween 20 solution only.

Pathogen Inoculation and Disease Rating:

All treatments were inoculated with specific compatible rice blast race IB-45 for Sakha 104 (5, 10, 15 and 20 days after treatments). Rice seedlings in each tray, were artificially inoculated with spore suspension (100 ml) adjusted to 5×10^4 spores/ml. Gelatin at 2.5 g/L was added to the spore suspension (Bastiaans, 1993) to enhance the adhesion of spores on leaf surfaces. Each isolate was sprayed with spore suspension using electrical spray gun. Inoculated plants were kept in a dew chamber for 16 h at 25°C under 100% relative humidity to facilitate fungal penetration and then transferred to a greenhouse (25 to 30°C, 80% relative humidity) for disease development. Seven days after inoculation, blast reaction was recorded according to the standard evaluation system using 0-9 scale (IRRI 1996).

Disease assessment:

Leaf blast infection percentage was assessed for each treatment by counting the number of infected leaves from 100 leaves. While, the total number of type 4 lesions on the infected leaves was used as criterion for severity of infection.

Damaged leaf area: The damaged leaf area for each treatment was calculated using this formula as follows:

$$\text{Damaged leaf area (DLA) \%} = \frac{\text{Lesion no.} \times \text{lesion size}}{\text{Leaf area}} \times 100$$

Lesion index %: This parameter compares the sporulating lesion number to the total lesion number according to **Yasuda *et al*., 2008 as follow**:

$$\text{Lesion index \%} = \frac{\text{No. of sporulating lesions}}{\text{Total no. of lesions}} \times 100$$

Area under disease progress curve (AUDPC):

To compare relative levels of blast disease progress for each treatment, data of leaf blast severity were converted to area under disease progress curve (AUDPC) with 5 days intervals. According to the formula described by Pandy and Merian (1989):

AUDPC = D ½ (X1 + Xn) + X2 + X3 + ……….. Xn-1

Where **X1, X2, X3 ……… Xn** = scores of blast severity at a constant intervals of D-days.

Agronomic traits: Data of plant height, flag leaf area, chlorophyll content, fresh and dry weight were taken after applying the final treatment. Flag leaf area (cm^2) was measured using a leaf area meter. These traits were used to follow the response to chemical inducer and its effect on plant growth.

Effect of the tested antioxidants on the linear growth of *M. grisea*:

Two phenolic antioxidants (salicylic acid, Bion (BTH) were used to study their direct and antifungal effects on the linear growth of *M. grisea* on BDA (Banana dextrose agar) plates. The solutions of effective concentrations (0, 4, 8 and 16 mM) of Salicylic acid and (0, 0.1, 0.3 and 1 mM) of Bion (BTH) were saturated in small filter paper and distributed in three positions in surrounding to disk (7 mm in diameter) which taken from the growing edge of 7-day-old colony of *M. grisea* to inoculate the prepared plates. For each treatment, four replicates were used. The plates were incubated at 28 C for 7 days. The linear growth of *M. grisea* in each treatment was measured.

Biochemical changes associated with disease resistance

The main objective of this assay is evaluate the role of PO and PPO in blast disease resistance interaction.

Crude enzyme preparation:

During 2010 season, the activities of oxidative enzymes were determined of 35-day rice seedlings , cv. Sakha 101, which is considered susceptible to rice blast disease and pretreated at 25 days with SA 4,6,8 mM ; H_2O_2 10,20,30% ; Bion (BTH) 1mM; tea compost. Enzymes extract was prepared as methods adopted by Maxwell and Betman (1967); rice leaves (4g) were ground with 0.01 M sodium phosphate buffer at 7.1 (pH) in the ratio of 1: 2 (w:v) in a china mortar. Triturated tissues were strained through 4 layers cheese cloth and filtrates were centrifuged at 3000 rpm for 20 min. at 6^oC. Leaf blast infection percentage under nursery conditions was assessed for each treatment by counting the number of infected seedlings from 100 tested ones.

a. Peroxidase (PO) assay:

The enzyme was determined according to the methods described by Allam and Hollis (1972) and Srivastava (1987) by measuring the oxidation of pyrogallol to purpurgallin in the presence of H_2O_2. The samples cuvette contained 0.5 ml of 0.1 M sodium phosphate buffer at pH 7.0, 0.3 ml enzyme extract, 0.3 ml of 0.05 M pyrogellol, 0.1 ml of 10% H_2O_2 and distilled water to bring cuvette contents to 3.0 ml. Po activity was expressed as changes in absorbance (mg protein 5 min/g sample) using a Spectrophotometer at 425 nm, and recorded at 0 to 25 min. intervals.

b. Polyphenol oxidase (PPO) assay:

The enzyme activity was determined according to the methods adopted by Matta and Dimond (1963). The reaction mixture contained 1.0 ml enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH 7.0, 10.0 ml of 0.001 M catechol and 3.0 ml distilled water. PPO activity was expressed as changes in absorbance (mg protein min/g sample) at 495 nm and recorded at 0 to3 min. intervals .In each determination, the control treatment (blank) contained all the chemical reagents except enzyme extract .

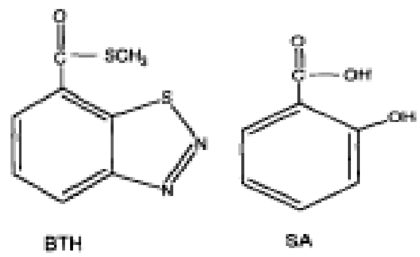

Figure 1: Structures of SAR-inducing Compounds, Comparison of the structures of two known plant activators: SA and BTH.

Statistical analysis: Data were subjected to analysis of variance (Gomez and Gomez, 1984), and means significantly differ were compared according to Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of different antioxidants on some rice blast disease parameters under greenhouse conditions:

In order to control this disease, the phenolic antioxidants were tested (BTH, salicylic acid, benzoic acid, Nicotinic acid, Cinnamic acid), H_2O_2 and Compost tea. The antifungal activity of the phenolic substances were investigated against *M. grisea* at different concentrations in vitro, as well as the efficacy of their induced application in controlling rice blast disease under greenhouse condition.

Under greenhouse condition, artificial inoculation was applied after four fixed periods (5, 10, 15 and 20 days after treatment (DAT)) from the application date of all antioxidants, concerning infection % of rice blast, there are a remarkable significant differences among all antioxidants compared with control. All antioxidants (Table 1) significantly reduced the infection percentage. Salicylic acid was the most effective antioxidants at concentration 8 mM, it recorded 4.9, 9.7, 28 and 45.5 % with 5, 10, 15 and 20 DAT, respectively. Also, Bion (BTH) at both concentrations 0.3 and 1.0 mM exhibited a significant reduction in infection %. The rest of antioxidants exhibited significant increase in infection % especially under late induced periods from 10-20 DAT. All antioxidants and compost tea reduced the infection percentage compared with untreated check especially with inoculation 5 DAT. The same trends were recorded with severity of infection under all treatment.

Under greenhouse conditions, spraying of Salicylic acid at 8 mM and Bion at 0.3 and 1.0 mM led to a significant reduction in disease severity and disease infection % on the plant leaves, Table 1.

Benzothiadiazole (BTH) has been described as exogenous elicitors of some plant defense compounds, polyphenols among them. Systemic acquired resistance induced by benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH) in rice against bacterial leaf blight (BLB) caused by Xanthomonas oryzae pv. oryzae was studied. Rice plants (IR 50) pre-treated with BTH showed resistance to a challenge infection with *Xanthomonas oryzae* pv. *oryzae*. About 50% reduction in disease intensity was observed in plants treated with BTH at 100 µg a.i./ml. Immunoblot analysis using barley chitinase antiserum revealed the induction of a 35 kDa chitinase in rice in response to treatment with BTH. The results indicate that the BLB resistance can be induced even in genetically susceptible cultivar through application of BTH, Babu *et al*., 2003.

Concerning the optimal period of induced resistance, artificial inoculation 5 DAT recorded the lowest infection percentage compared with the inoculation after 10 to 20 days. With Salicylic acid, artificial inoculation 5 DAT to 15 days with both Salicylic acid and Bion led to a significant reduction in disease infection percentage and severity. While the resistance was remarkably sharply decreased after 15 days from induced resistance treatment with all antioxidants. The optimal induce period of Benzoic acid , Nicotinic acid , H2O2 , Compost tea and Cinnamic acid was continued from 5 to 10 days only, Table 1.

For Area under disease progress curve (AUDPC), the use of AUDPC as a criterion for blast infection, reflecting disease severity in time is easier than using individual scores for the evaluation of disease development. So, results revealed that all antioxidants treatments showed lower AUDPC values than those untreated treatments. Treatment with salicylic acid at 8 mM recorded the lowest AUDPC, 409.48 compared by the control 1304.24, followed by Bion at 0.3 and 1.0, Table 1.

This induced resistance rarely leads to complete control of pathogens following subsequent inoculation, but rather results in, for example, a reduction in lesion numbers and size, Kuc, 1982.

Foliar application with BTH significantly reduced blast infection percentage, severity and AUDPC of rice seedlings. The optimal concentration of salicylic acid and BTH treatment for inducing SAR was at 8 and 0.3-1.0 mmol/L, respectively, the proper time of treatment was at the 5th day prior to inoculation with *M. grisea*, and the period of induced resistance could last for 15 d or more. Moreover, the disease severity of the upper untreated leaves markedly reduced after the second leaf was treated with the same concentrations of both salicylic acid and BTH. This indicates that salicylic acid and BTH significantly induced the SAR of rice seedlings to blast disease. The results also show that treatment of rice the 2nd leaf with salicylic acid and BTH enhanced the rice SAR to *M. grisea* at 3rd-4th leaf stage of rice seedlings, Table (1) and Figs. (1 & 2).

Concerning hypersensitive reaction HR, Responses of susceptible rice cultivar Sakha104 to *Magnaporthe grisea* after treatment with antioxidants was investigated. Pretreatment with salicylic acid and Bion, prior to pathogen inoculation rapidly triggered the hypersensitive reaction (HR), resulting in microscopic cell death around the pathogen, so significantly increased resistance to *M. grisea*. Both salicylic acid and BTH exhibited the highest number of HR spots on the upper leaves as symptoms of induce resistance, 67.7 and 49.3 spots, respectively Table (1). Acquired resistance was expressed in new leaves emerging after SA and BTH treatment of older leaves compared with untreated plants. Exogenously applied SA did not protect the treated 2 leaf but caused SAR in the younger leaves that were emerging after the treatment (Table 1). Although the systemic protection was only partial, it was significant compared with control. These data may indicate that SA and BTH are sufficient for generating a systemic signal leading to SAR in other parts of the plant.

Most R gene-triggered resistance is associated with a rapid defense response, termed the hypersensitive response (HR). The HR results in a localized cell and tissue death at the site of infection, which prevent the further spread of the infection (Hammond-Kosack and Jones, 1997). The local response, however, results in a non-specific systemic acquired resistance (SAR) throughout the plant. The defense responses activated include an oxidative burst, which can lead to cell death, thereby trapping the pathogen in dead cells; changes in cell wall composition that can inhibit pathogen penetration; and synthesis of antimicrobial compounds such as phytoalexins, Heath, 1998.

Plants suffer various types of inevitable exogenous stresses, such as pathogen attacks, insect herbivory, and abiotic environmental stress. To survive such unfavorable conditions, plants have evolved unique hormonally regulated self-protection systems. Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) contribute to responses against biotic stresses by influencing various signaling pathways that have complex networks of synergistic and antagonistic interactions (Kunkel and Brooks, 2002). In incompatible interactions between plants and pathogenic microorganisms, plants recognize the avirulence gene products of individual pathogens using specific receptors, the R gene products. This interaction causes, at the infection site, a burst of reactive oxygen species (ROS), the rapid induction of a hypersensitive response (HR) involving regulated cell death, and the expression of pathogenesis-related (PR) genes (Durrant and Dong, 2004). Incompatible interactions lead to the onset of rapid local defense responses, including ion fluxes, activation of kinases, and accumulation of reactive oxygen intermediates. Hypersensitive response (HR)-associated cell death around the infection site limits the spread of the pathogen to surrounding cells, and also evokes a systemic defense response, which is mediated through the production of secondary signal molecules, such as salicylic acid (SA), jasmonic acid (JA), ethylene (ETH), and nitric oxide (NO). Plant defense responses are accompanied by the induced expression of pathogenesis-related (PR) genes locally at the point of infection, as well as systemically (Mittler, *et al*. (1997) and McDowell and Dangl (2000). The rapid induction of these genes serves as an indicator of incompatible interactions (Bent, 1996; Dangl, *et al*. 1996 and Hammond-Kosack and Jones 1996). Systemic acquired resistance (SAR), the most well characterized systemic defense response, confers resistance to a broad spectrum of pathogens and can be induced by treatment of SA or its analogs, such as benzothiadiazole (BTH), (Friedrich, *et al*.1996 and Gorlach, *et al*.1996) and methyl- 2,6-dichloroisonicotinic acid (INA), Delaney, (1997). Application of one of these SAR-inducing chemicals to a susceptible host prior to pathogen infection leads to very rapid induction of HR and PR gene expression upon pathogen infection, resulting in no or reduced disease (Katz, *et al*. 1998 and Lawton, *et al*.1996).

Table (1): Influence of different antioxidants on infection of rice blast disease parameters of Sakha104.

No.	Treatment	Infection %				Mean	Severity of infection				Mean	HR spots 5DAT/ 10 leaves	AUDPC
		5	10	15	20		5	10	15	20			
1	Control	43.7	83.9	75.0	79.4	70.50	108.3	199.5	253.1	547.1	277.0	5.3	1065.24
2	Control+ tween 0.05%	37.9	72.6	64.6	79.3	63.60	115.4	187.2	280.5	750.0	333.3	6.3	1304.24
3	Bion (BTH) 0.1 mM	22.9	39.7	64.5	77.0	51.03	117.9	172.5	171.5	529.5	247.9	26.0	938.24
4	Bion 0.3 mM	8.1	32.0	40.3	55.9	34.08	116.7	179.2	137.3	304.3	184.4	36.7	662.9
5	Bion 1.0 mM	4.9	9.7	28	45.5	22.03	87.5	125	96.0	238.2	136.7	49.3	491.77
6	Salicylic acid 8 mM	6.7	13.8	12.9	30.6	16.00	58.3	80.5	91.6	210.5	110.2	67.7	409.48
7	Benzoic acid 8 mM	4.8	60.9	90.8	100	64.13	75.0	242.4	168.0	517.3	250.7	16.3	986.93
8	Nicotinic acid 8mM	6.9	65.8	79.1	79.6	57.85	100.0	183.1	148.9	541.7	243.4	21.0	937.87
9	H_2O_2 30%	6.6	47.6	69.8	79.1	50.78	91.7	117.7	144.5	503.6	214.4	21.3	825.33
10	Compost tea 100%	7.9	51.8	57.4	67.2	46.08	87.5	111.5	127.2	575.0	225.3	12.5	879.95
11	Cinnamic acid 8mM	8.6	44.7	57.5	71.8	45.65	100.0	123.1	197.1	782.7	300.7	19.3	1191.17
	Mean	14.45	47.50	58.17	69.58		96.2	156.5	165.1	500.0			
	L.S.D. 5 % time of application	8.53					48.90					2.339	
	Treatment	14.14					81.09						
	Treat. × time	28.28					162.19						

HR, Hypersensitive reaction, AUDPC, Area under disease progress curve

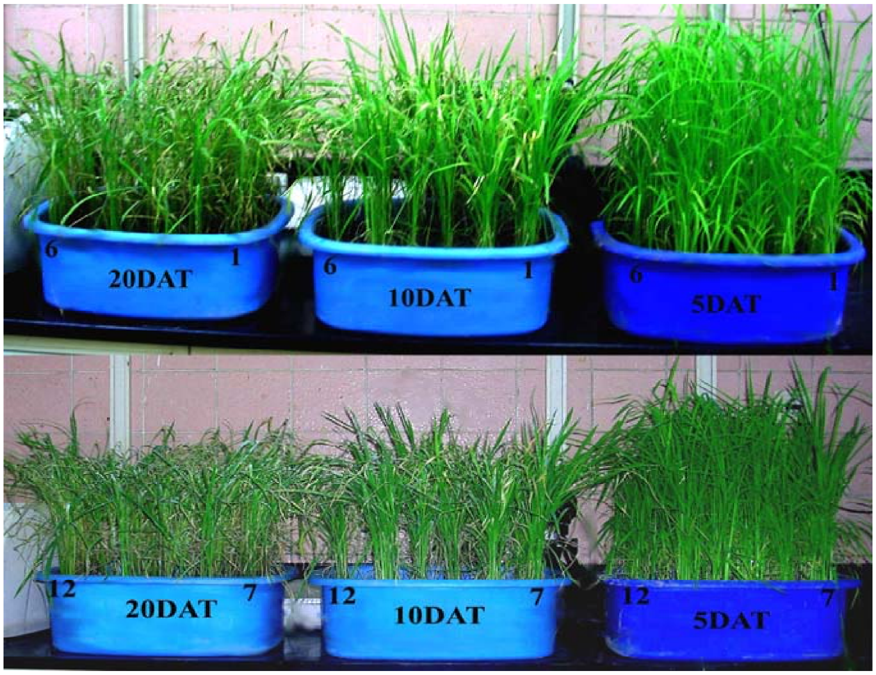

Fig. (2): Rice blast development on Sakha104 under different antioxidants and periods of artificial inoculation after treatment (days after treatment, DAT).

Table (2): Effect of different effective concentrations of Bion (BTH) and salicylic acid on physiological characters of *Magnaporthe grisea*.

No.	Treatment	Linear growth cm	No. of spores /ml	Spore germination%	Appresorium formation %
1	Control	8.3	9.7*	86.7	76.7
2	Bion (BTH) 0.1 mM	8.2	11.0	83.3	78.3
3	Bion 0.3 mM	8.2	9.3	85.0	75.0
4	Bion 1.0 mM	8.3	11.3	86.7	80.0
5	Salicylic acid 4.0 mM	8.2	11.7	85.0	76.7
6	Salicylic acid 8.0 mM	8.3	10.0	86.7	78.3
7	Salicylic acid 16.0 mM	8.3	12.3	88.3	76.7
	L.S.D. 5 %	0.399	2.355	6.337	9.742

*No. of spores×10^{-4}

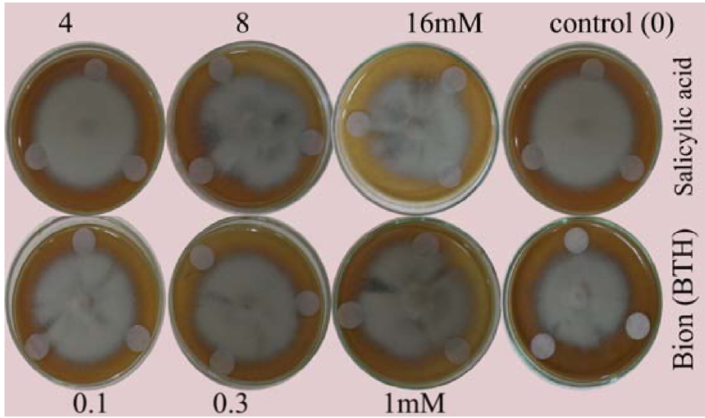

Fig. (3): Effect of phenolic antioxidants; Salicylic acid and Bion on linear growth of Magnaporthe grisea PDA plates.

Effect of some antioxidants on blast fungus growth under laboratory conditions:

Laboratory studies indicated that BTH and Salicylic acid had no suppressive and fungicidal effect on the mycelial linear growth, spore germination, sporulation and appressorium formation of *Magnaporthe grisea* in vitro there is no significant differences among all treatments Table (2) and Fig. (3). SA and BTH with all concentrations had no antifungal activities so, they had no significant protective effect. So, it could be concluded that the protective effect observed is not as a consequence of antimicrobial activity of SA and BTH but it is due to their defense-triggering responses. SA acid and BTH as a synthetic chemical are capable of inducing resistance against rice blast disease, but without direct antifungal activity at the effective concentration. Abiotic inducers like benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), salicylic acid (SA) have shown the effectiveness to induce systemic acquired resistance (SAR) in a variety of plants against a wide range of pathogens without possessing direct antimicrobial activity in their chemical properties in vitro or in planta.(Yoshida *et al*. 1990,Gorlach *et al*., 1996, Lawton *et al*., 1996 and Cole 1999).

Results in Table (3) revealed that SA and BTH treatments had significant protective effect when it applied 5-15 days before inoculation with *Magnaporthe grisea* fungus. So, it could be concluded that the protective effect observed is not a consequence of anti-microbial activity of SA and BTH but due to their defense-triggering responses. This effect has also been seen in other plants. An increment in protective effect was apparent when rice plants were inoculated 5 days after SA treatment, but it was more distinct when inoculation was performed 15 days after SA and BTH treatment.

Table (3): Influence of different antioxidants on protection % of rice against blast disease on Sakha104.

No.	Treatment	Protection %				
		5	10	15	20	Mean
1	Control	-	-	-	-	-
2	Control+ tween 0.05%	-	-	-	-	-
3	Bion (BTH) 0.1 mM	88.79	52.68	14.00	3.02	**39.62**
4	Bion 0.3 mM	84.67	83.55	46.27	29.60	**61.02**
5	Bion 1.0 mM	89.02	88.44	62.67	42.70	**70.71**
6	Salicylic acid 8 mM	84.21	83.55	82.80	61.46	**78.01**
7	Benzoic acid 8 mM	84.90	27.41	0.00	0.00	**28.08**
8	Nicotinic acid 8mM	81.92	21.57	0.00	0.00	**25.87**
9	H2O2 30%	80.32	43.27	6.93	0.38	**32.73**
10	Compost tea 100%	81.92	38.26	23.47	15.37	**39.76**
11	Cinnamic acid 8mM	80.32	46.72	23.33	9.57	**39.99**
	Mean	**84.01**	**53.94**	**28.83**	**18.01**	

All treatments; Bion, Salicylic acid, Benzoic acid, Nicotonic acid, H2O2, compost tea and Cinnamic acid had 80% protective effect more than control 5 days after treatment. The best protective effect (89.0 %) with BTH treatment 5 days before inoculation at concentration 0.1 mM, also, SA exhibited the longest protective period which persistent from 5-15 days with more than 80 %. While, many spreading lesions appear on the leaves of treated control plants, sparse lesions were seen on the leaves of SA- and BTH-treated plants. Treatment with SA and BTH resulted in over 80% protection from pathogenic infection, confirming the effect of SA and BTH against rice blast disease. In the previous report, it was suggested that SA and BTH is related to the resistant reaction of rice plants, because the treatment of SA, as well as with BTH, increased the ratio of minute brownish lesions (HR lesions) to spreading lesions (Yoshida *et al*. 1990). Resistance induced by these agents (resistance elicitors) is broad spectrum and long lasting, but rarely provides complete control of infection, with many resistance elicitors providing between 20 and 85% disease control. There also are many reports of resistance elicitors providing no significant disease control. In the field, expression of induced resistance is likely to be influenced by the environment, genotype, and crop nutrition, Walters *et al*., 2005.

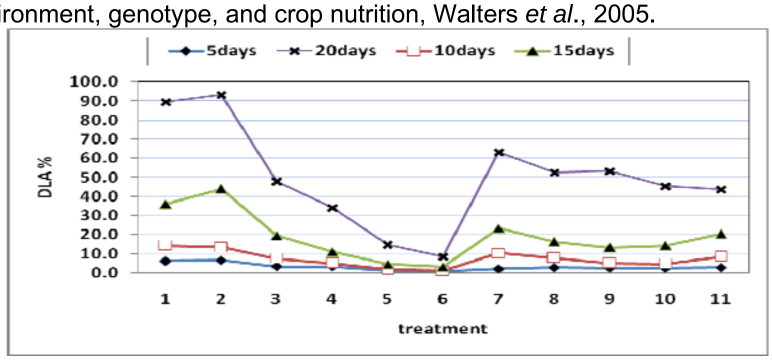

Fig. (4): Damaged leaf area % (DLA) of Sakha104 due to infection with *M. grisea* after 5-20 days of spraying with different antioxidants.

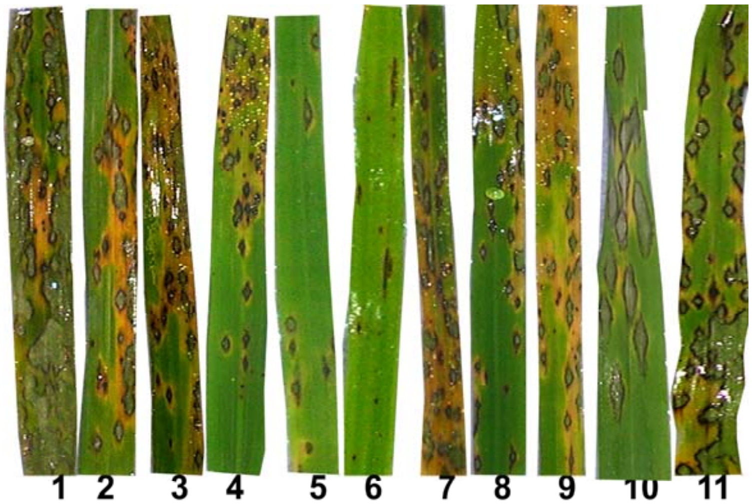

Fig. (5): Damaged leaf area % (DLA) of Sakha104 due to infection with *M. grisea* after 20 days of spraying with different antioxidants.

For damaged leaf area % (DLA%), treatments of rice plants No. 4, 5 and 6 (**Table 1**) with Salicylic acid at 8 mM and Bion 0.3 and 1.0 mM resulted in the least minimum damaged leaf area % compared with control which have the highest maximum distractive area (Figs. 4 & 5). For the proper time to induce resistance was at 5th day earlier than inoculation with *M. grisea*, it exhibited the minimum damaged leaf area % with all treatments and the period of induced resistance could last for 15 d or more. Moreover, the disease severity of the upper untreated leaves markedly deceased after the second leaf was treated with the same concentrations of both salicylic acid and BTH. The fact that all of these chemicals are effective in rice and can improve the resistance to blast disease. Acquired resistance of rice blast can be efficiently triggered by different chemicals like SA and BTH. The broad-spectrum activity of BTH, conferring protection against bacterial, fungal and viral diseases strongly suggests an indirect mode of action via activation of plant defense mechanisms, Babu *et al*., 2003.

Table (4): Influence of different antioxidants on sporulation of M. grisea and rice lesion index % of Sakha104.

No.	Treatment	10 days				15 days			
		No. of lesion/ 10 leaves	No of sporulating lesion/ 10 leaves	lesion index %	No of spores/ ml	No. of lesion/ 10 leaves	No of sporulating lesion/ 10 leaves	lesion index %	No of spores/ ml
1	Control	45.1	35.1	77.83	17*	148.3	138.3	93.26	45*
2	Control+ tween	53.1	45.1	84.93	27	170.3	165.3	97.06	51
3	Bion (BTH) 0.1 mM	27.1	10.1	37.27	4	127.3	112.3	88.22	17
4	Bion 0.3 mM	19.1	3.1	16.23	2	110.3	55.3	50.14	7
5	Bion 1.0 mM	16.1	2.1	13.04	2	86.3	30.2	34.9	6
6	Salicylic acid 8.0 mM	18.1	3.1	17.13	1	52.3	15.2	29.1	4
7	Benzoic acid 8.0 mM	50.1	35.1	70.06	15	174.3	168.3	96.56	34
8	Nicotinic acid 8.0 mM	41.1	13.1	31.87	4	165.3	163.3	98.79	15
9	H2O2 30%	48.1	33.1	68.81	18	150.3	145.3	96.67	42
10	Compost tea 100%	23.1	11.2	48.48	6	87.3	52.3	59.91	12
11	Cinnamic acid 8.0 mM	19.1	11.2	58.64	9	160.3	140.3	87.52	10
	L.S.D. 5 %	0.488	0.488		0.051	0.977	0.977		0.215
	1%	0.664	0.664		0.069	1.328	1.328		0.292

***× 10^{-4} days after seeding.**

Concerning lesion index which reflected the comparison of sporulating lesion with the total lesions on infected leaves and sporulation capacity, the control exhibited the highest lesion index that means 84.93 % of lesions have vigorously produced spores. On the other hand, the least lesion index was recorded from treatments with BTH and SA 13.04-17.13 %. Also, all treatments reduced the lesion index value compared with control in 10 days after treatment. The treatments of SA and BTH 1.0 mM reduced the lesion index % 29.1 and 34.9 after 15 days of inoculation compared with control, respectively. While the rest of treatments exhibited the same results as in untreated treatments. Several previous studies have reported that BTH could activate the development of systemic acquired resistance and result in disease resistance against virus and fungal attack in different crops (Gorlach *et al.,* 1996, Benhanou and Belanger, 1998, Anfoka, 2000) In addition to the hypersensitive response that blocks the local growth of an infecting pathogen, a secondary defense response can be triggered that renders uninfected parts of the plant resistant to a variety of normally virulent pathogens (Ryals *et al.,* 1996). This broad spectrum disease resistance is known as systemic acquired resistance (SAR). Salicylic acid accumulates after pathogen infection in a wide range of plants, and has been found to accumulate in systemic tissue following pathogen infection and is closely associated with the development of SAR (Ryals *et al.,* 1996). In addition, exogenous application of SA or its analogues, such as 2, 6- dichloroisonicotinic acid (INA) and benzothiadiazole (BTH) has been shown to induce SAR (Kessmann *et al.,* 1994; Lawton *et al*., 1996). Associated with the SA accumulation and the onset of SAR is the induction of a group of pathogenesis related (PR) genes, which encode small secreted or vacuole targeted proteins with antimicrobial properties (Ryals *et al.,* 1996; Durrant and Dong, 2004). In rice, however, the role of SA in R gene-mediated and induced resistance, is not clear. Endogenous SA levels in rice are significantly higher than in dicotyledonous plants and SA levels do not increase in the upper leaves of plants in which systemic resistance to blast fungus is induced by *P. syringae* D20 pre-inoculation of lower leaves (Silverman et al. 1995). Because exogenous application of SA poorly induces PR (pathogenesis-related) gene expression or resistance to blast fungus infection, Yang et al. (2004) suggested that SA modulates the plant’s redox balance to protect it from oxidative stress, rather than acting as the defense signal for inducing a resistance response. Recent studies have showed that salicylic acid (SA)- and ethylene (ET)/jasmonic acid (JA)-mediated signaling pathways, which are critical to activation of defense responses of dicot-plants against biotrophic and necrotrophic pathogens, respectively, also play important roles in rice disease resistance responses (Glazebrook, 2005). In rice, distinct mechanisms might be required for its defense responses against different pathogens because different types of defense-responsive genes were found to be involved in resistance to bacterial blight and fungal blast diseases (Wen *et al.*, 2003; Ahn *et al*., 2005). However, it was also found that the SA and ET/JA-mediated signaling pathways in rice may operate in concert and share some common components or biochemical events (Qiu *et al*., 2007; Ding et al., 2008). SA is synthesised in plants either via the PAL pathway, or via isochorismate synthase (ICS) (Wildermuth et al., 2001). Evidence suggests that SA synthesised through ICS has an important role in plant defense against pathogens, and that it is required for PR1 gene expression and SAR defence responses. However, SA also potentiates cell death in response to particular pathogens or fungal elicitors (Dempsey *et al.,* 1999). As plants that are defective in ICS gene expression still exhibit cell death when infected with necrotising pathogens, SA that potentiates plant cell death is probably synthesised through PAL (Wildermuth *et al.,* 2001).

Table (5): Effect of different antioxidants on some agronomic traits of Sakha104 under greenhouse condition.

No.	Treatment	Leaf area Cm^2 -10 leaves	Plant height cm	Chlorophyll content (SPAD)- 15 DAT	Fresh weight (g)	Dry weight (g)	Chlorophyll content - 20 DAT
1	Control	22.64	39.00	30.83	0.875	0.203	32.26
2	Control+ twin	21.78	39.0	27.10	0.750	0.170	32.93
3	Bion (BTH) 0.1 mM	19.52	38.66	27.00	0.970	0.214	33.53
4	Bion 0.3 mM	14.81	35.66	29.00	1.018	0.218	34.53
5	Bion 1.0 mM	22.20	37.33	25.60	0.987	0.218	34.60
6	Salicylic acid 8.0 mM	15.26	35.0	29.30	1.180	0.285	32.23
7	Benzoic acid 8.0 mM	16.93	37.50	30.20	1.433	0.314	37.50
8	Nicotinic acid 8.0 mM	17.77	40.50	29.70	1.455	0.294	35.56
9	H2O2 30%	19.947	37.16	27.40	1.326	0.278	35.03
10	Compost tea 100%	16.84	40.66	28.00	1.178	0.235	36.46
11	Cinamic acid 8.0 mM	26.36	36.66	25.30	1.020	0.225	33.00
	L.S.D. 5 %	9.95	4.100	0.355	0.260	0.054	1.623
	1%	13.52	5.57	0.482	0.353	0.073	2.21

Effect of different antioxidants on some agronomic traits under greenhouse conditions:

Regarding to the response of some growth characters of Sakha 104 rice cv. to antioxidants, no significant differences in leaf area and plant height were found among all treatments and control. On the other hand, the application of the previous treatment led to a significant increase in the chlorophyll content especially after 20 days from inoculation. Finally, all treatments led to significant increase in both fresh and dry weight.

Table (6): Effect of some antioxidants on some agronomic traits of Sakha101 under greenhouse condition.

No.	Treatment	Leaf area Cm^2-10 leaves	Plant height cm	Chlorophyll content (SPAD)- 15 DAT	Fresh weight (g)	Dry weight (g)
1	Control	9.18	25.17	36.17	1.41	0.301
2	Control+ twin	14.17	24.17	32.23	1.36	0.310
3	Bion (BTH) 0.1 mM	17.76	26.17	33.30	1.46	0.322
4	Bion 0.3 mM	17.61	28.83	36.47	1.29	0.250
5	Bion 1.0 mM	21.08	30.17	36.57	1.57	0.378
6	Salicylic acid 8 mM	22.01	31.83	39.77	1.71	0.409
7	Benzoic acid 8 mM	19.01	35.00	39.43	1.28	0.274
8	Nicotinic acid 8 mM	21.05	37.33	37.63	1.36	0.280
9	H2O2 30%	22.57	33.00	38.73	1.19	0.247
10	Compost tea 100%	21.50	30.33	38.50	1.56	0.324
11	Cinamic acid 8mM	19.52	28.33	36.57	1.33	0.289
	L.S.D. 5 %	3.338	3.415	1.661	0.306	0.063
	1%	4.537	4.642	2.257	0.416	0.085

For growth characters of Sakha 101, applications of the previous treatments led to a significant increase in leaf area, plant height, chlorophyll content, fresh and dry weight of rice leaves compared to control. In general, Sakha 101 rice cultivar exhibited the highest response to applications of all antioxidants compared with other cultivar Sakha 104 that have a weak response of growth characters to antioxidants application.

Effect of different antioxidants on blast infection and enzymes activities under nursery conditions:

Since, oxidative enzymes of PO and PPO play as a key of plant disease resistance. Results in Table(7) showed that, PO activity with both SA 4mM and BTH 1.0 mM increased continuously in 0-25 min. intervals and markedly significant increase ,although PO activities reach to be maximum at 20-25 min. at which it was higher than that of the control and other treatments. SA had the highest activities in period intervals followed by BTH. SA 4 mM exhibited the lowest infection percentage and Bion compared with control under nursery conditions. All treatments significantly reduced the blast infection % compared with control. Tea compost and H_2O_2 showed no significant increase in PO activity with all time intervals while the other treatments showed PO activities only in 10-20 min. compared to control. PO oxidases phenolics to highly toxic quinones hence had been assigned a role in disease resistance (Vidhyasekaran, 1988), polymerization of phenolic compounds during wall modification (Kerby and Samervlle,1989) and catalysis of terminal steps in lignin biosyntheses, in presence of H_2O_2 (Zhang *et al.* 1997).Moreover , Achou *et al*, (2004) ,Bokshi *et al*.(2003) ,Bovie *et al.* 2004) added that, BTH and SA could be enhance resistance as activation of signal transduction pathway and prime key defense genes such as PR proteins ,*viz.* peroxidase . the results supported by finding of Chakraborty *et al*. (2005), who showed that SA elicitation of PO in tea showing resistance reduction against blight disease .Also, Morris *et al.* (1998), Malolepsza (2005) and Phuntumart *et al*. (2006) added that, SA, BTH reduced infection development of maize leaf spot, tomato gray mold, cucumber anthracnose, respectively ,and rice blast (Gorlach *et al.* (1996).

Table (7): Peroxidase activity in rice ,cv. Sakha 101 under nursery and natural infection condition.

Treatment	Infection % Nursery seedlings (35- day)	Peroxidase activity unit /mg protein /min.					
		0	5	10	15	20	25
SA 4mM	5 d	0.534g	2.577d	2.908h	3.252g	3.446i	3.425h
6mM	6 d	0.380e	1.167b	1.334ef	1.381d	1.463f	1.453e
8mM	8 d	0.168c	1.089b	1.355f	1.450e	1.527g	1.514f
H2O2 10%	9 cd	0.185c	0.749a	1.033a	1.094a	1.174a	1.164b
20	16 bc	0.193c	1.036b	1.269d	1.292c	1.350d	1.320d
30	18 b	0.063a	0.994b	1.313e	1.364d	1.452e	0.907a
Compost tea 100%	19 b	0.136b	0.947ab	1.193c	1.241b	1.291b	1.256c
Bion (BTH) 1 mM	6 d	0.258d	2.083c	2.869g	3.088f	3.221h	3.231g
Control	52 a	0.407f	1.161b	1.127b	1.279c	1.322c	1.322d

Data in Table (8) showed that, PPO activity has gradually decreased during intervals 0 to3 min. Spraying by SA 4mM recorded the highest enzyme activity in all intervals and markedly significant increase in activity. So, SA 4 mM exhibited the lowest infection percentage and Bion compared with control. Although, SA 6mM (0-2 min.), SA8mM (1,2min.) 10 % (0, 2, 3 min.) and tea compost (1, 2 min) recoded high enzyme activity compared to control. All treatments significantly reduced the blast infection % compared with control after 10 days from treatments with antioxidants under nursery conditions. The lowest concentration of H_2O_2 10 % reduced the blast infection more than the highest ones. The results were supported by Mohammadi and Kazemi (2002), they found that PPO is involved in the oxidation of polyphenol into quinones (antifungal compounds) and lignifications of plant cells during pathogen penetration so it's increasing means of plant defense and resistance.

SA and BTH may play an important role in mediating defence-gene activation and systemic acquired resistance (SAR) in rice. In response to pathogen attack, plants produce a highly specific blend of SA,JA, and ET, resulting in the activation of distinct sets of defense-related genes (Koornneef and Pieterse, 2008; Bari and Jones, 2009). It is thought that this so-called signal signature, which varies greatly in quantity, timing, and composition according to the type of attacker encountered, plays a primary role in the orchestration of the plant's defense response and eventually determines the specific nature of the defense response triggered (Rojo et al.,2003; De Vos *et al*., 2005; Mur *et al*., 2006).

Table (8): Polyphenoloxidase activity in rice, cv. Sakha 101 under nursery and natural infection condition.

Treatment	Infection % Nursery seedlings (35 days)	Polyphenoloxidase activity unit /mg protein /min.			
		0	1	2	3
SA 4mM	5 d	1.054i	0.992i	1.011i	1.013h
6mM	6 d	0.661g	0.665h	0.630h	0.625f
8mM	8 d	0.596e	0.605g	0.571f	0.586e
H_2O_2 10%	9 cd	0.724h	0.524d	0.604g	0.633g
20	16 bc	0.522c	0.491c	0.485c	0.486d
30	18 b	0.438b	0.433b	0.415a	0.412a
Compost tea 100%	19 b	0.563d	0.551f	0.549e	0.446c
Bion (BTH) 1 mM	6 d	0.412a	0.427a	0.431b	0.428b
Control	52 a	0.612f	0.534e	0.545d	0.625f

It is commonly accepted that SA promotes resistance against pathogens with a biotrophic life- style, whereas JA and ET act as positive signals in the activation of defenses against necrotrophic pathogens and herbivorous insects (Thomma *et al*., 2001; Rojo *et al*., 2003; Glazebrook, 2005).

Salicylic acid (SA) is a natural inducer of disease resistance in some dicotyledonous plants. Rice (*Oryza sativa* L.) seedlings had the highest levels of SA among all plants tested for SA content (between 0.01 and 37.19 pg/g fresh weight). The second leaf of rice seedlings had slightly lower SA levels than any younger leaves. However, leaf SA levels in 28 rice varieties showed a correlation with generalized blast resistance, indicating that SA may play a role as a constitutive defense compound. Biosynthesis and metabolism of SA in rice was studied and compared to that of tobacco. Rice shoots converted Cinnamic acid to SA and the lignin precursors P-coumaric and Ferulic acids, whereas benzoic acid was readily converted to SA. The data suggest that in rice, SA is synthesized from Cinnamic acid via Benzoic acid. In rice shoots, SA is largely present as a free acid; however, exogenously supplied SA was converted to P-O-D-glucosylSA by an SA-inducible glucosyltransferase (SA-CTase). A 7-fold induction of SA-CTase activity was observed after 6 h of feeding 1 mM SA. Both rice roots and shoots showed similar patterns of SA-CTase induction by SA, with maximal induction after feeding with 1 mM SA. Silverman *et al.* (1995).

BTH was the most potent inducer of both resistance and gene induction. BTH is being developed commercially as a novel type of plant protection compound that works by inducing the plant's inherent disease resistance mechanisms. Both of SA and BTH treatment resulted in almost quantitative resistance of the youngest leaf under severe disease pressure.

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تـأثيربعض المـواد الكيماويـة المسـتحثة للمقاومـة وتوقيـت تطبيقهـا علـي الأصـابة بمرض لفحة الأرز علي الصنفين سخا١٠١ و سخا ١٠٤

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[١] **معهد بحوث أمراض النباتات ـ مركز البحوث الزراعية ــ مصر**
[٢] **مركز البحوث و التدريب في الأرز- معهد بحوث المحاصيل الحقلية ـ مركز البحوث الزراعية**
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يعتبر مرض اللفحة *Magnaporhte grisea* أكثر أمراض الأرز خطورة حيث يسبب خسارة معنوية للمحصول. تؤدي طبيعة التنوع وتغير السلالات لفطر اللفحة إلي كسر المقاومة لأعلي الأصناف أنتاجية مثل سخا ١٠١ وسخا ١٠٤. والمكافحة الكيماوية ليست فعالة دائما كما أنها ضارة علي البيئة وصحة الأنسان. بعض مضادات الأكسدة والأحماض العضوية مثل البيون (BTH) بتركيزات ٠.١ و ٠.٣ و ١ ملليمول ، حمض السلسيلك ٨ ملليمول وحمض البنزويك ٨ ملليمول ، والنيكوتنك ٨ ملليمول ، وفوق أكسيد الهيدروجين ٣٠ % ومستخلص الكمبوست ١٠٠ % تم رشه مباشرة علي المجموع الخضري قبل العدوي بفطر اللفحة وذلك لاستحثاث المقاومة في أوراق الأرز. تحت ظروف العدوي الصناعية في الصوبة ، أجريت العدوي بعد أربع فترات ثابتة وهي ٥ ، ١٠ ، ١٥ و ٢٠ يوم بعد المعاملة لكل من مضادات الأكسدة. أحدثت كل مضادات الأكسدة ومستخلص الكمبوست خفضت في نسبة الإصابة باللفحة مقارنة بالمعاملة الغير معاملة وخاصة عند العدوي بعد ٥ أيام من المعاملة. بالنسبة لنسبة الإصابة يوجد أختلافات معنوية ملحوظة بين كل مضادات الأكسدة مقارنة بالكنترول. كل مضادات الأكسدة تخفض نسبة الإصابة معنويا. وكان حمض السلسيلك أكثر مضادات الأكسدة فاعلية عند تركيز ٨ ملليمول حيث سجل ٤.٩ ، ٩.٧ ، ٢٨ و ١٥.٥ % عند ٥ ، ١٠ ، ١٥ و ٢٠ يوما بعد العدوى وأيضا البيون عند تركيز ٠.٣ ، ١ ملليمول حيث حقق انخفاضا معنويا في الإصابة. بينما حققت باقي مضادات الأكسدة حققت زيادة معنوية في الإصابة وخاصة بعد الفترات المتأخرة من العدوي من ١٠- ٢٠ يوما. كانت الفترة المثلي لإستحثاث المقاومة هي ٥ أيام من العدوي الصناعية حيث حققت أقل نسبة أصابة مقارنة بفترة ١٠-٢٠ يوما. يمتد التأثير بالمعاملة بكل من حمض السلسيلك والبيون من ٥-١٥ يوما من العدوى بينما يحدث أنهيار ملحوظ للمقاومة بعد ١٥ يوم من أستحثاث المقاومة. وكانت الفترة المثلي لإستحثاث المقاومة بالنسبة لحمض البنزويك والنيكوتينك وفوق أكسيد الهيدروجين والسيناميك ومستخلص الكمبوست أستمرت من ٥-١٠ أيام فقط. حققت المعاملة بالسلسيلك أقل مساحة تحت منحني المرض(٤٠٩.٤٨) مقارنة بالمقارنة (١٣٠٤.٢٤) ويليها المعاملة بالبيون عند تركيز ٠.٣ و ١ ملليمول. والبيون والسلسيلك ليس لها تأثير مثبط لنمو الفطر والتجرثم وعضو الأختراق لفطر *Magnaporthe grisea* . أستمر نشاط أنزيم البيروكسيداز من صفر- ٢٥ دقيقة مع المعاملة بالسلسيلك ٤ ملليمول والبيون وكانت أكثر من المقارنة وباقي المعاملات. وحقق السلسيلك النشاط الأعلي ويليه البيون. لم يحقق مستخلص الكمبوست وفوق أكسيد الهيدروجين أختلاف في نشاط الأنزيم مع أختلاف الفترة الزمنية بينما المعاملات الأخري أظهرت اختلافا في النشاط بأختلاف الفترة من ١٠-٢٠ دقيقة. أظهر أنزيم البولي فينول أوكسيداز تناقصا تدريجيا في الفترة من صفر إلي ٣ دقائق . سجل الرش بالسلسيلك ٤ ملليمول أعلي نشاط أنزيمي تحت كل الفترات الزمنية. أدت كل معاملات مضادات الأكسدة إلي زيادة معنوية في مساحة ورقة العلم وطول النبات ومحتوي الكلوروفيل والوزن الرطب والجاف مقارنة بالغير معامل. وعامة أظهر الصنف سخا ١٠١ أستجابة أعلي من الصنف سخا ١٠٤ الذي أظهر أستجابة ضعيفة في النمو للمعاملة بمضادات الأكسدة.

قام بتحكيم هذا البحث

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