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Enhancing Tomato Plant Resistance Against *Tobacco Mosaic Virus* Using Riboflavin

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Farida M Mahmoud*, Ahmed M Bondok, Walaa Khalifa, Fawzy Abo El-Abbas
Plant Pathology Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68, Hadayek Shoubra, 11241, Cairo, Egypt

*Corresponding author: Faridamohamed@agr.asu.edu.eg

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Abstract

Plant systemic resistance induced by natural product is an alternative technique of disease management. Riboflavin (vitamin B2) usually used as abiotic elicitor to improve the plant immunity against different pathogens. This work aimed to explore the efficiency of three riboflavin concentrations (0.5, 2.5, and 5 mM) to enhance resistance toward *Tobacco Mosaic Virus* (TMV) in tomato plants. Our results showed that exogenous application of 2.5mM riboflavin, 5 days before virus challenge was the most effective concentration, which provided a reduction in disease incidence and disease severity by 80% and 75%, respectively. Furthermore, obtained results were confirmed by using DAS-ELISA test, which showed that concentration 2.5 mM of riboflavin decreased the virus concentrations by 46.4% in treated- inoculated plants. It was remarked that exogenous application of 2.5 mM riboflavin showed a positive effect on some plant growth parameters either in presence or absence of TMV challenge. The plant height and number of leaves per plant were significantly improved in treated- inoculated plants about 30% and in compared to the infected control 78%. In time course investigation, 2.5mM riboflavin treatment reduced the virus symptoms particularly at 9th day, where after the symptoms become evident. In addition, expression of phenylalanine ammonia-lyase (PAL) and pathogenesis-related protein (PR10), which are the markers of systemic acquired resistance (SAR), were rapidly increased in inoculated-treated tomato from 1-3 days after treatment. Moreover, the results of the biochemical changes analysis revealed that, the levels of the defense markers including peroxidase (PO), and polyphenol oxidase (PPO) were increased four days after of treatment and reached maximum levels at 8 days in the treated- inoculated plants. In conclusion, it could be suggesting that riboflavin exerted a great influence on TMV disease, as indicated by reduction of disease symptoms as well as enhancement of biochemical changes in plant defense against infection with TMV.

Keywords: Tomato, *Tobacco Mosaic Virus*, Riboflavin, Systemic acquired resistance, Biochemical analysis

1 Introduction

Tobacco Mosaic Virus (TMV) is well-studied virus (Rybicki 2015, An et al 2019) which cause a destructive crop loss in many vegetable and ornamental crops throughout the world (Lv et al 2017). TMV infects commercially grown tomato worldwide, and seriously inflict the quality and yield (Waziri 2015, Zhao et al 2017). For the avoidance of TMV disease, different strategies were applied which couldn't have been effective. Over the years, an unconventional management strategy has been applied in an effort to mitigate the extensive economic damage caused by TMV (Srivastava et al 2015). One solution might be focused on systemic acquired resistance (SAR), which plays very important role in the improving of resistance of plant against different agents (Kong et al 2018, Guo et al 2020). SAR mentions to a distinct signal transduction pathway that is usually triggered through exogenous application of chemicals, leading to a long-term resistance to a wide variety of pathogenses (Dempsey and Klessig 2012, Lucas et al 2013). SAR mechanism has been

associated with improved, more efficient activation of response and primed resistance to stimulating biotic and abiotic stress (Conrath 2011). Numerous researches reported vitamins and their essential role towards the development of a safe and sustainable resistance in plant (Aranega-Bou et al 2014, Boubakri et al 2016).

Riboflavin (vitamin B_2) is recognized as an essential bio-sensitized product in plants and many microorganisms. It contributes many ethnobotanical uses and pharmacological activities (Deng et al 2014), and modulate several different physiological processes such as growth of plant and defense responses (Taheri and Tarighi 2011). Several studies have illustrated the effect of riboflavin in improving disease resistance against a broad-spectrum pathogen (Conrath 2011). Riboflavin is participated in the anti-oxidation (Perumal et al 2005), and peroxidation processes (Nazarul et al 2006), which both enhance the reactive oxygen reactions and other plant defense mechanisms (Nie and Xu 2016). Moreover, treatment with riboflavin induces defensive responses in plants instead of directly inhibition of pathogens growth (Aver'yanov et al 2000, Taheri and Höfte 2006). Recently, riboflavin activates PR-genes in *Arabidopsis* and induces SAR to pathogens (Boubakri et al 2013, Deng et al 2014).

With this background the present study aims to evaluate the riboflavin for its resistance eliciting efficiency against TMV infection in tomato plants as a classical SAR system as well as an investigation some of the important biochemical and molecular events involved in the process.

2 Materials and methods

2.1 Plant materials

Twenty-five-days old seedlings of *Solanum lycopersicum* var. Super Strain B were transplanted individually into 20cm pots, containing mixtures of clay and sand (1: 3 v/v). The plants were kept in the greenhouse at 23-25°C and photoperiod of 16h. They were irrigated as needed and fertilized as usual.

2.2 Source of the virus

The used strain of TMV was kindly provided by Virus Lab., Department of Agriculture Microbiology, Faculty of Agriculture, Ain shams University. Inoculum consisted of symptomatic leaves of TMV-infected tobacco were ground (1/3 w/v) in 25mM sodium phosphate buffer (pH 7.0) containing 0.002 M EDTA and celite (0.1 mg ml^{-1}) as abrasive (Chen et al 2017).

2.3 Inducing chemical and experimental set up

Riboflavin (Biomatic Corporation) was diluted in distilled water containing 0.02% v/v Tween-20 to a series of concentrations (0.5, 2.5, 5mM) and sprayed onto leaf surfaces of 3-4 leaf stage tomato plants until run-off. Sterile distilled water containing 0.02% v/v Tween-20 was used as negative control. Plants were kept at 22ºc and 65% humidity. Five days later, plants were divided into groups and treated as follows: (1) plants treated with sterilized distilled water only [Healthy control]; (2) plants inoculated with TMV; (3) plants treated with 0.5mM riboflavin; (4) plants treated with 0.5mM riboflavin and inoculated with TMV; (5) plants treated with 2.5mM riboflavin; (6) plants treated with 2.5mM riboflavin and inoculated with TMV; (7) plants treated with 5mM riboflavin and (8) plants treated with 5mM riboflavin and inoculated with TMV. Each group consisted of ten replicates. All experimental plants were arranged in completely randomized block design and the experiments were carried out in two successive growing seasons.

2.4 Plant growth characters

At the end of the experiment, plants were harvested and some morphological parameters such as plant height and leaf number per plant were assessed.

2.5 Assessment of disease resistance

The level of TMV resistance was evaluated based on disease incidence and severity of symptoms. Disease incidence was assessed as the percentage of plants that showing typical virus symptom 30 days post inoculation. The disease severity was evaluated using a scale according to Wang et al (2009) while the severity index was estimated using the following formula as described by Yang et al (1996).

$$Disease\ Severity\ (\%) = \frac{\sum(disease\ grade \times number\ of\ plants\ in\ each\ grade)}{(Total\ number\ of\ plants\ \times highest\ disease\ grade)} x100$$

Leaf samples were taken 3 weeks later for measuring virus concentration using bioassay and ELISA techniques.

2.6 Quantification of TMV infectivity

Virus infectivity has been quantified in treated-infected plants and infected control plants by extracting their crude sap and inoculation on *Nicotiana glutinosa* leaves. Number of developed local lesions, seven days later on inoculated plants were counted and calculated for the using the following formula as described by Prasad et al (1995).

% Inhibition = (number of local lesions of treated sample / number of local lesions of control) x 100

2.7 Determination of TMV concentration

TMV accumulation in the leaves was determined by DAS-ELISA (Clark and Adams 1977) using polyclonal antibodies (Bioreba AG, Switzerland) according to the provided instructions. Samples were considered positive if the ELISA absorbance value was greater than twice for comparable negative control samples (Córdoba-Sellés et al 2007).

2.8 Time course of SAR by riboflavin

The concentration of 2.5mM riboflavin was the most effective one, so this concentration was used for this study. Tomato plants were inoculated with TMV after 0 to 21 days of riboflavin treatment. Control plants were similarly treated with sodium acetate buffer (20mM, pH 5.2). Symptoms and inhibition percentage of virus infection were daily recorded till 21th day later to express resistance induced. Moreover, the virus concentration was determined during the time course using DAS-ELISA.

2.9 Gene expression analysis of *PAL* and *PR10*

Tomato leaf samples (100mg) were collected at 0, 3, 6 and 12 hours after riboflavin treatments (2.5Mm). Total RNA was extracted by total RNA purification kit (Jena Bioscience, Germany) according to the manufacturers' instructions. Presence or absence of pathogensis related protein (PR10) - and PAL- genes was established by RT-PCR, using pair of specific primers (*PAL*-Forward, 5′-AAGCTGATGTTCGCGCAGTTCT-3′, and *PAL*-reverse, 5′- AAACCATAGTCCAAGCTCGGGT-3′), (*PR10*-Forward, 5′-AAGGAGATGTTCTTGGAGACAAACTTG-3′, and *PR10*- reverse, 5′- AGCGTAGACAGAAGGATTGGCG-3′). The expression of the gene of interest was normalized, relative to that of the housekeeping actin genes (*Actin*- Forward, 5′-TCATACGGTCAGCAATAC-3; *Actin*- reverse, 5-ATGTGGATATCAGGAAGGA-3. In order to obtain cDNA, mRNA was reverse transcribed using a one-step RTPCR kit (QIAGEN, Hilden, Germany). PCR reactions were subjected in a thermal Eppendorf master cycler (T100TM thermal cycler, BIO-RAD) under the following conditions: reverse transcription at 50 ºC for 30 min, followed by denaturation at 94ºC for 15 min, 30 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s, and a final extension of 72°C for 1 min. The RT-PCR products were checked through electrophoresis on 1.5% agarose gel and ethidium bromide stained bands were visualized under UV light.

2.10 Biochemical changes associated with riboflavin treatment

Peroxidase (PO), and polyphenol Oxidase (PPO) activities were estimated spectrophotometric ally using the appropriate wavelength for each enzyme. Samples were taken of healthy control, infected control, treated plant, and treated- infected plants at different period 0, 4, 8, 9, 10, and 13 days after inoculation. Crude enzyme was extracted according to Mofunanya et al (2016).

Peroxidase activity was assayed according to Wang's method (Wang 2006). PO activity was analyzed by measuring oxidation level of guaiacol at 470 nm per mg protein per minute, using a spectrophotometer (Hitachi U-1800, Japan). Each sample was tested 3 times.

Meantime, Polyphenol oxidase activity was determined according Ngadze et al (2012) in which 1 ml of leaf extracts of each control plant were mixed with 3 ml of freshly prepared reaction mixture containing 0.05 M buffer sodium phosphate and 0.1 M catechol solution (pH 6.5). The absorbance of the mixture was measured after 3 min at a wavelength of 420 nm. PPO activity is presented as the change in Unit mg^{-1} $protein^{-1}$ min^{-1}.

2.11 Statistical analysis

All the experiments were performed twice. There were presented as mean ±SE of at least three independent replicates for each determination. Analyses of variance were carried out using ANOVA. Least significant difference (LSD) was employed to test for significant difference between treatments at P-value < 0.05 (Gomez and Gomez 1984).

3 Results

3.1 Plant growth characters

Initial symptoms were observed on the infected tomato plants as discoloration and mosaic symptoms 20 days after inoculation. However, these symptoms appeared on riboflavin treated plants 30 dpi. Riboflavin treatments have generally enhanced the growth of both non-inoculated and TMV-inoculated plants, with maximal effect to 2.5mM riboflavin. In absence of TMV, riboflavin applied at 2.5mM significantly increase plant height by 38.59 and as compared to healthy control **Fig 1.** Number of leaves per plant 0.5 and 2.5mM riboflavin treated plants were also increased by about 42 and 72% compared with healthy control **Fig 2**. In absence of TMV, riboflavin applied at 2.5mM significantly increase Leaves number by 78.04 and as compared with healthy control **Fig 2**.

The infected plants showed substantial reductions of 20.35% in their height compared to non-inoculated plants (healthy control) **Fig 1**. However, 0.5 and 2.5mM riboflavin treated showed the same significant increase in the plant height of TMV-inoculated plants by 30.39 and compared to infected control plants **Fig 1**. Moreover, Leaf number per plant was significantly increased by 78.04 in TMV-infected plants grown at 2.5mM riboflavin compared with infected control **Fig 2**.

3.2 Assessment of disease resistance

Elicitors are a common feature of induced resistance to disease, allow enhancement of active defense mechanisms against different stresses (Chakraborty and Acharya 2017). Previous studies reported the efficiency of riboflavin to improve the plant immunity against different pathogens (Boubakri et al 2016). In the present study, the potential activity of three riboflavin concentrations (0.5, 2.5, 5mM) was assessed to enhance the resistance of tomato plants against TMV.

Data in **Fig 3** showed the effect of treatment with different concentrations of riboflavin on TMV disease incidence 30 days after inoculation with TMV. Riboflavin treating plants with 0.5 and 2.5mM significantly ($P \leq 0.05$) reduced TMV incidence by about 60 and 80%, respectively, as compared to infected control plants **Fig 3**. Whereas, disease severity was decreased by 62.5, 75, and 25% in plants sprayed with 0.5, 2.5, and 5mM riboflavin, respectively as compared to the infected control **Fig 4**.

3.3 Quantification of TMV infectivity

As shown in **Fig 5**, all TMV crude sap extracted of treated plants exhibited different degrees of reduction in local lesions number formed on *N. glutinosa*, relative to TMV crude sap extracted from infected control. Treated plants (0.5 and 5mM) showed a remarkable reduction in infection with TMV reached to 37.5 %, and 16.67%, respectively. However, 2.5mM riboflavin treated plants exhibited excellent reduction in TMV activity with decreased number of local lesions (87.5%). It was very clear that 2.5mM riboflavin played an important role for reducing TMV activity rather than other concentrations **Fig 5**.

3.4 Determination of TMV concentration

Virus concentration assessed by DAS-ELISA revealed that, TMV accumulation was greatly reduced in all treated- infected plants but it was not completely suppressed **Fig 6**. TMV quantitation data from two experiments indicated that the 0.5 and 2.5mM riboflavin treatments typically caused a decrease in TMV concentrations by 44 and 61.18%, respectively, as compared to the infected control **Fig 6**.

3.5 Time course of SAR by riboflavin

As shown in **Fig 7**, application of 2.5mM riboflavin before TMV challenge led to increase the plant resistance gradually from the 1st day. It reached a plateau between 6^{th} and 8^{th} day, peaked after 9^{th} day and finally declined gradually till the 21^{th} day of challenge **Fig 7**. Disease incidence and disease severity were decreased in treated- inoculated plants by 33.3 and 20% at 9^{th} day, respectively **Fig 7**. The same trend of reduction in disease symptoms of riboflavin treated plants was confirmed by DAS-ELISA, which revealed a significant inhibition in the virus concentrations at 9^{th} day **Fig 8**.

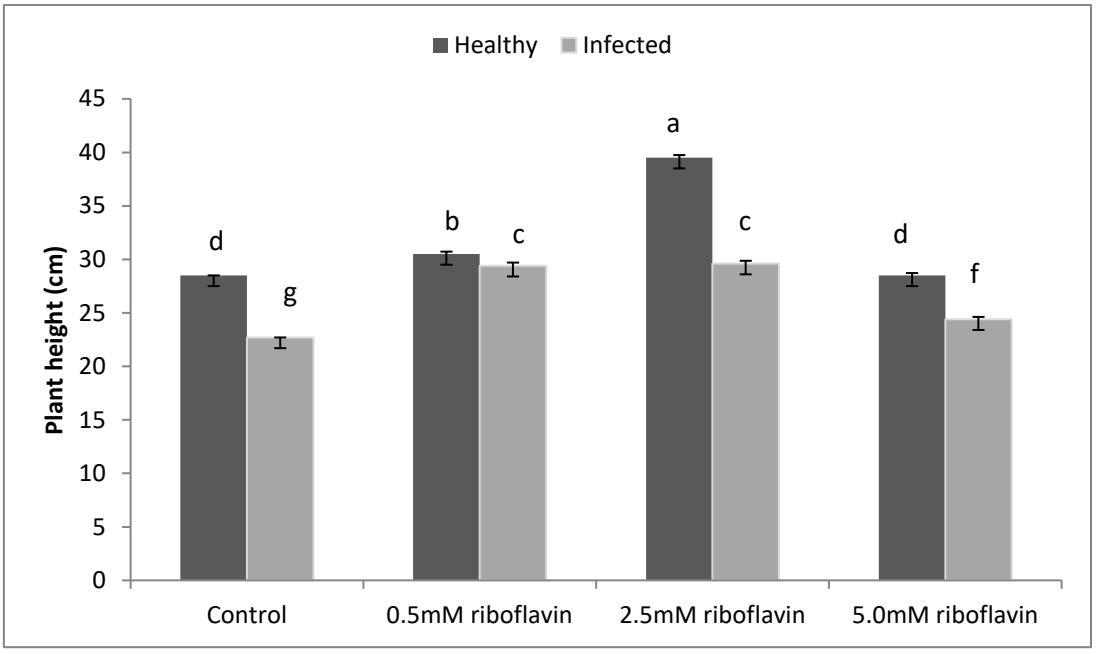

Fig 1. Plant height of tomato plants in response to riboflavin in deferent concentration and were inoculated 5days later. Ten plants were used per treatment and data obtained 30 days after inoculated. The same letter is not significant at p≤ 0.05and standard error of mean calculated

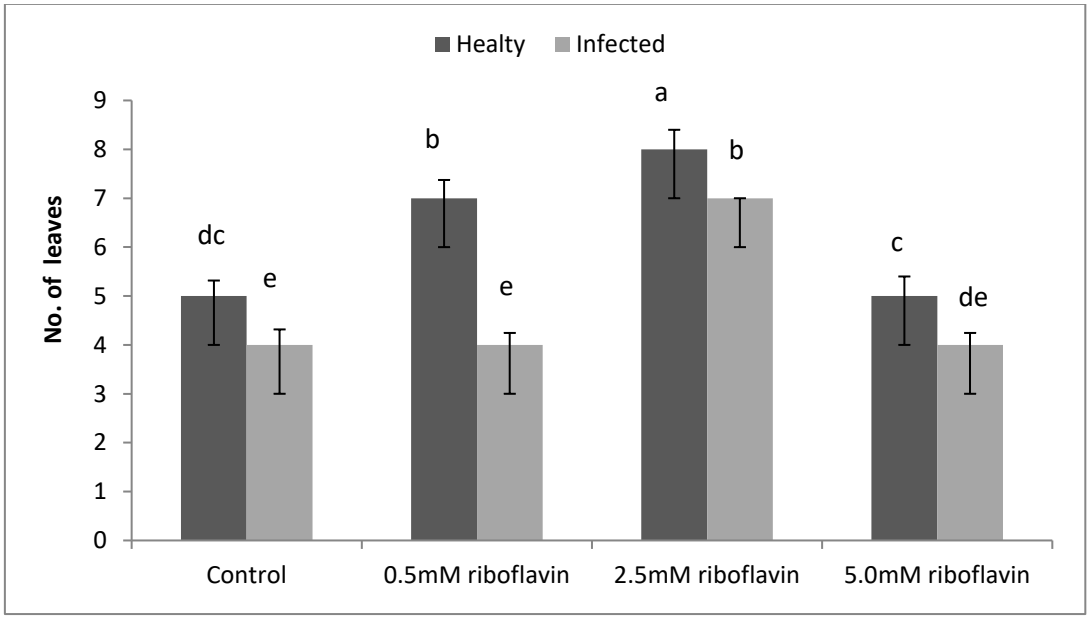

Fig 2. Number of leaves of tomato plants in response to riboflavin in deferent concentration and were inoculated 5days later. Ten plants were used per treatment and data obtained 30 days after inoculated. The same letter is not significant at p≤ 0.05and standard error of mean calculated

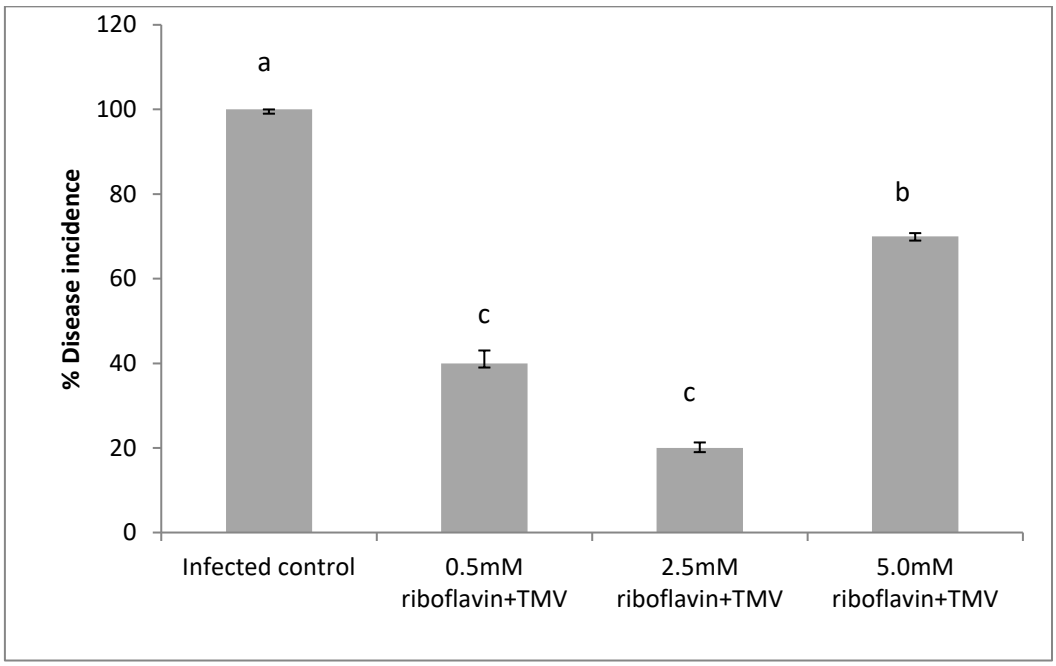

Fig 3. TMV incidence on tomato plants 35 day after challenge as affected by different concentration with riboflavin. Ten plants were used per treatment. The same letter is not significant at p≤ 0.05and standard error of mean calculated

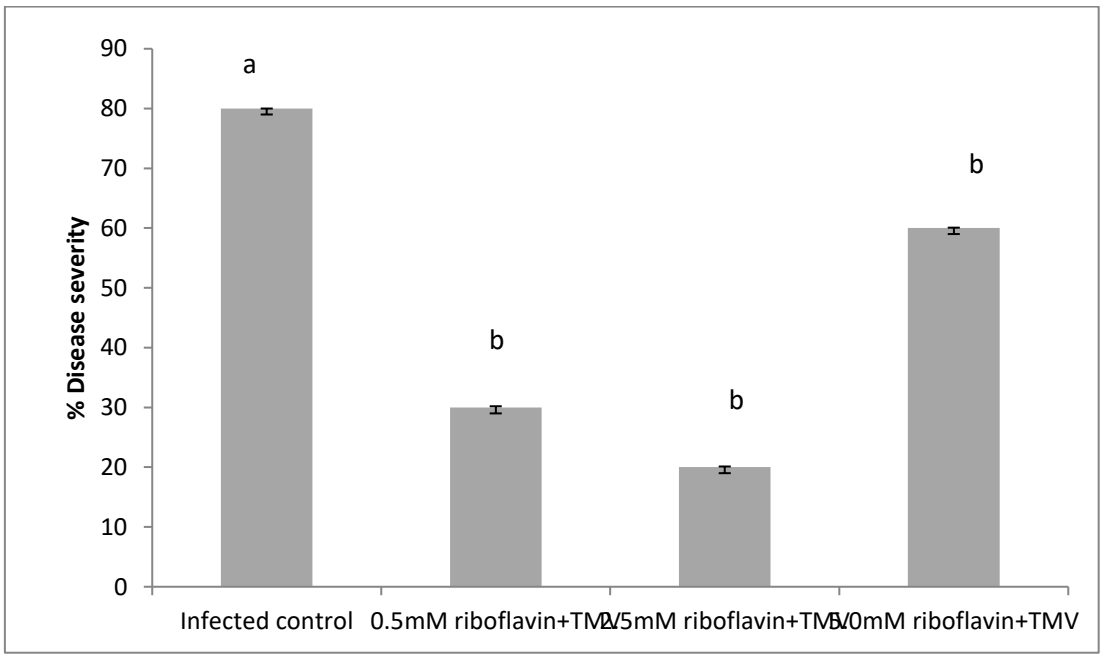

Fig 4. TMV severity on tomato plants 35 day after challenge as affected by different concentration with riboflavin. Ten plants were used per treatment. The same letter is not significant at p≤ 0.05and standard error of mean calculated

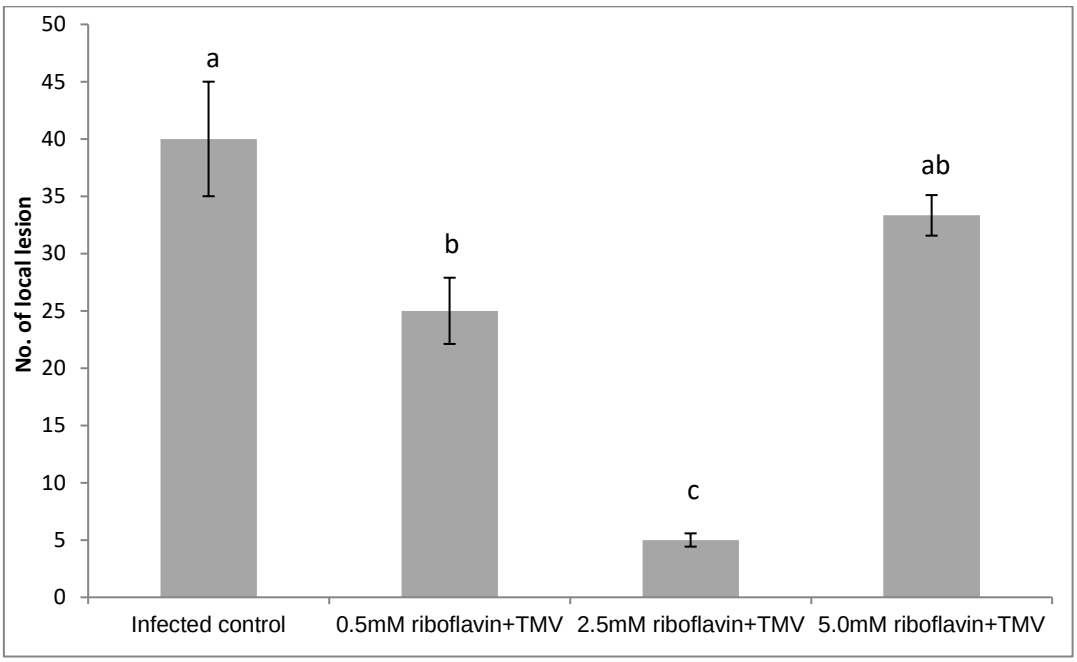

Fig 5. Effect of riboflavin on TMV infectivity according to formation of local lesion on *N. glutinosa* plant. Samples to prepare the infection sap were collected from treated tomato plants at 14 days after inoculation with TMV. Each experiment was repeated twice. Five plants were used per treatment and average number of local lesions was determined from 10 leaves each for treated. The same letter is not significant at p≤0.05and standard error of mean calculated

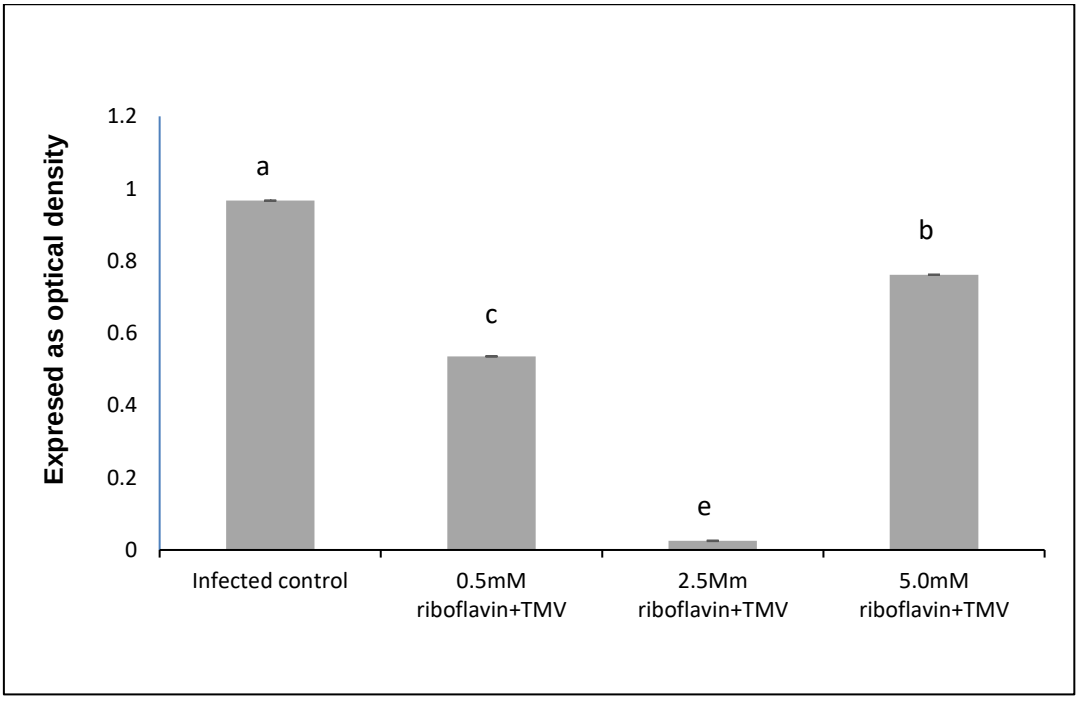

Fig 6. Effectiveness of riboflavin on virus concentration at 14 days after inoculation in tomato plants using DAS-ELISA. Each experiment was repeated twice. Ten plants were used per treatment and the same letter is not significant at p≤ 0.05 and standard error of mean calculated

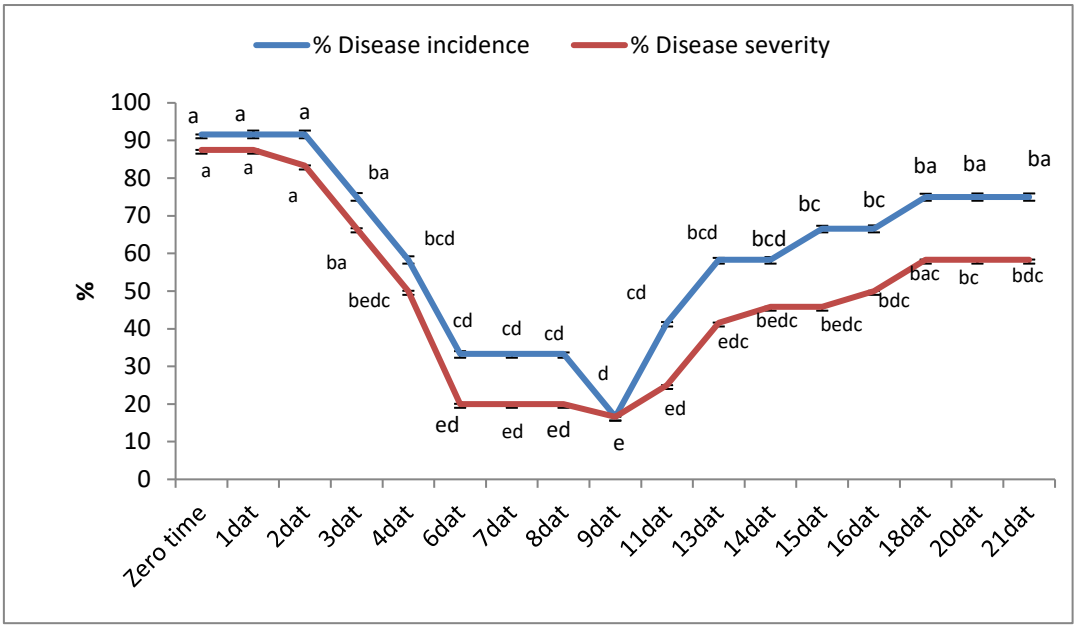

Fig 7. Time course riboflavin (2.5mM) on TMV development infection and severity. Ten plants were used per treatment and data obtained at 35 day after challenge. The same letter is not significant at p≤ 0.05 and standard error of mean calculated

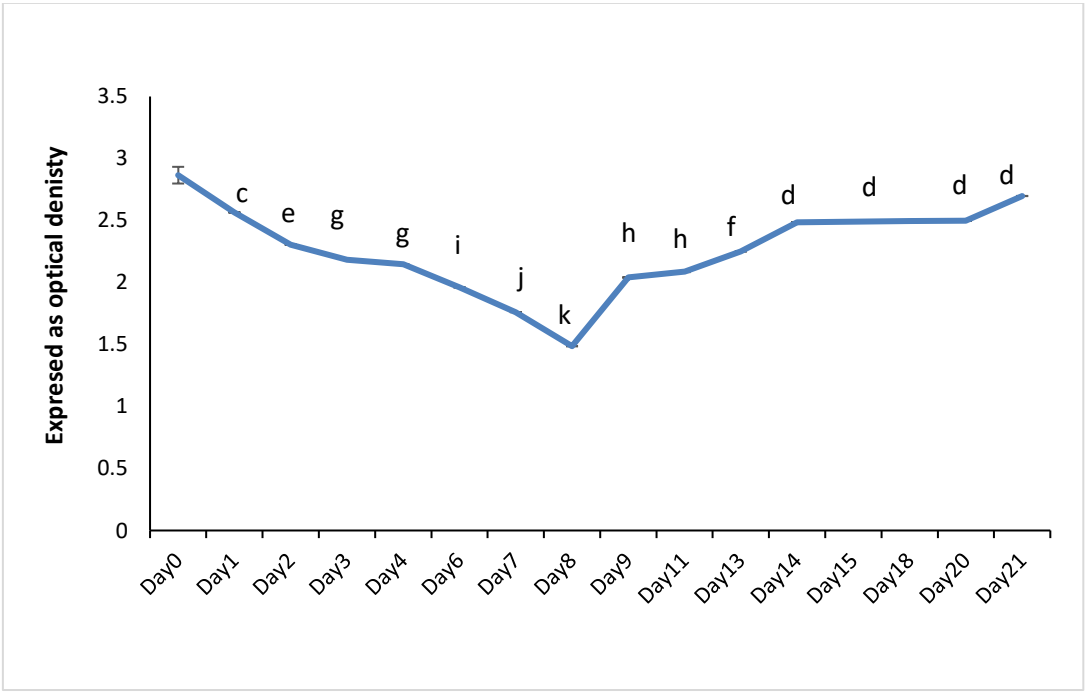

Fig 8. Time course riboflavin (2.5mM) on TMV concentration at 14 days after inoculation in tomato plants using DAS-ELISA. Each experiment was repeated twice. Ten plants were used per treatment and the same letter is not significant at p≤ 0.05and standard error of mean calculated

3.6 Gene expression analysis of *PAL* and *PR10*

Data illustrated in **Fig 9** showed that 2.5mM riboflavin treatment markedly induced the accumulation of PAL-mRNA in the beginning 3 hours and raised at 6 h, followed by a slightly decrease at 12 h. Similarly, *PR10* accumulation was notable increased at 3 and 6 h and waked at 12h after treatment **Fig 9**. Interestingly, the accumulation of *PR-10*, one of the members of PR10 proteins, has displayed significantly phosphorylation, which enhanced resistance against TMV in *Capsicum annuum* as previously recorded (Ali et al 2018). Whereas, there was no remarkable different in the mRNA level of *actin* at any time points.

3.7 Biochemical changes associated with riboflavin treatment

Results in **Fig 8** revealed that, PO activity was improved and significantly peaked at maximum level at 8th day in both non-infected and TMV-infected plants after the riboflavin treatment **Fig 10**. This increased activity remained stable till 9th day, and then was followed by sharp declining trend during the time course. Peroxidase activity in treated – inoculated plants was 1.6 and 1.4 times higher than those of healthy and infected controls at the 8th day, respectively **Fig 10**.

Likewise, accumulation of polyphenol oxidase (PPO) activities was gradually increased in the first 4 days, and then raised sharply till 8 day in riboflavin treated plants in response to TMV infection. After nine days of elicitation, enzyme activities were gradually diminished. Results in **Fig 9** denote further that, PPO was slightly activated in treated- non inoculated plants over healthy and infected control plants during the time course **Fig 11**.

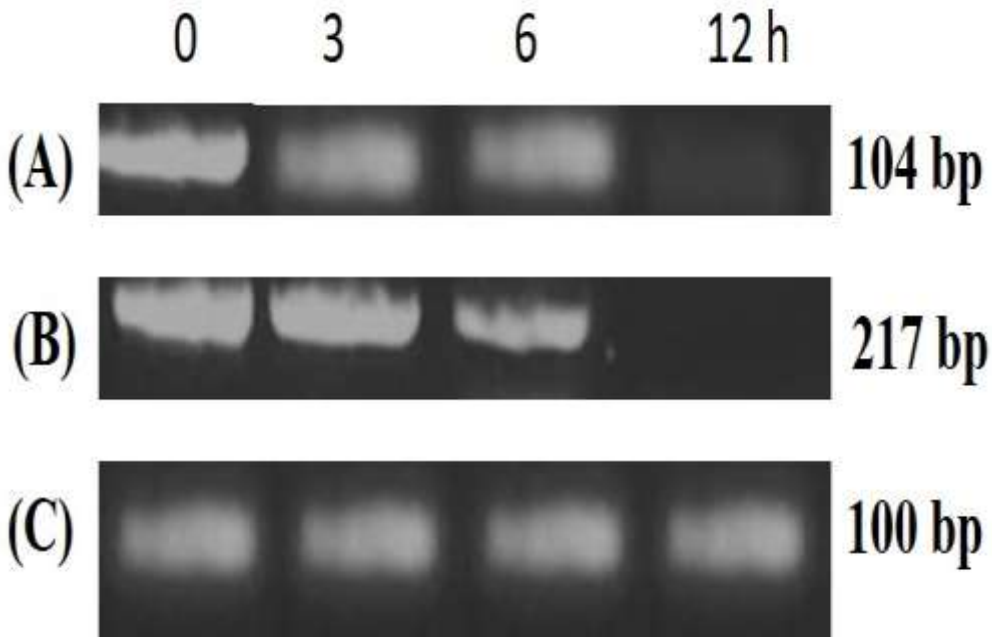

Fig 9. RNA expression level of *PAL* and *PR10* genes in tomato plants at different time points (0, 3, 6, 12 h) after exogenous application with 2.5 mM riboflavin. A, Transcription level of PAL at expected molecular weight (104 bp). B, Transcription level of expression of PR-10 at expected molecular weight (217 bp). C, Expression of actin gene as housekeeping gene.

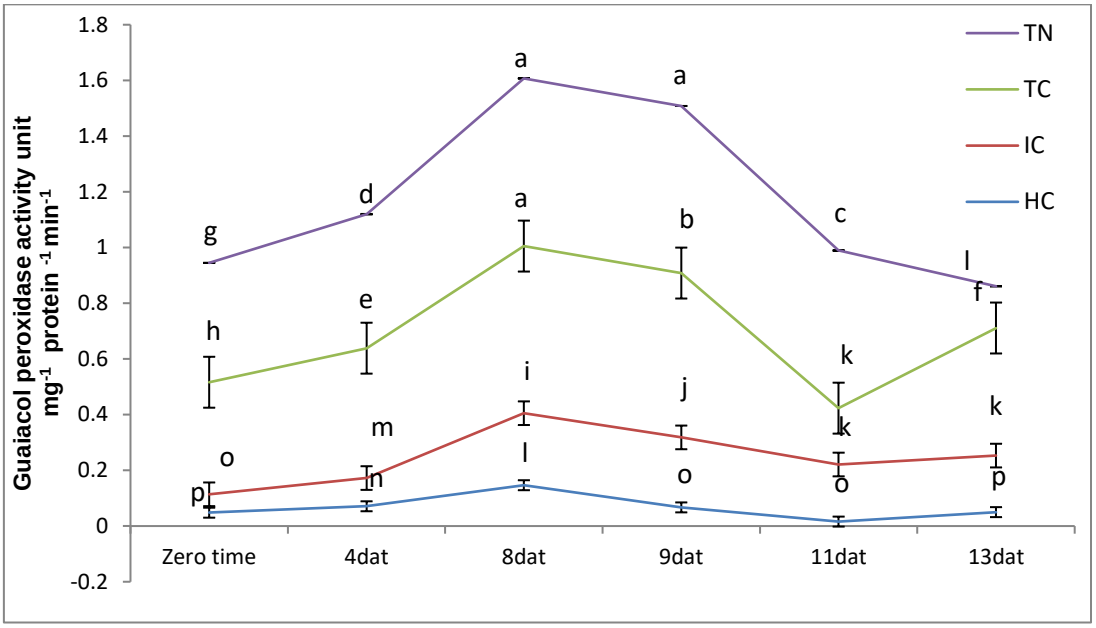

Fig 10. Effect of treatment with 2.5mM of riboflavin on peroxidase activity in tomato plants. HC: Healthy control, IC: Infected control (non- treated inoculated plants), TC: treated (2.5Mm) non-inoculated, TN: treated inoculated plants with 2.5mM of riboflavin, Optical density at 470 nm. Data obtained at least from 10 plants / treatment. The same letter is not significant at p≤ 0.05 and standard error of mean calculated

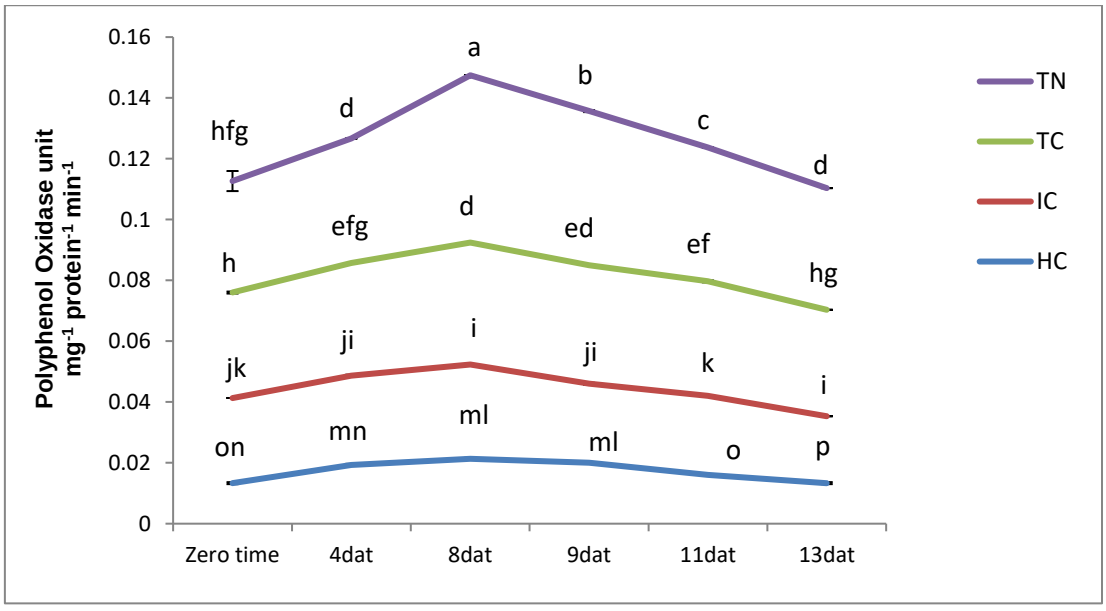

Fig 11. Effect of treatment with 2.5mM of riboflavin on Polyphenol Oxidase activity in tomato plants. HC: Healthy control, IC: Infected control (non- treated inoculated plants), TC: treated (2.5Mm) non-inoculated, TN: treated inoculated plants with 2.5mM of riboflavin, Optical density at 420 nm. Data obtained at least from 10 plants/ treatment. The same letter is not significant at p≤ 0.05 and standard error of mean calculated

4 Discussion

Elicitors are a common feature of induced resistance to disease, allow enhancement of active defense mechanisms against different stresses (Chakraborty and Acharya 2017). Previous studies reported the efficiency of riboflavin to improve the plant immunity against different pathogens (Boubakri et al 2016). In the present study, the potential activity of three riboflavin concentrations (0.5, 2.5, 5mM) was assessed to enhance the resistance of tomato plants against TMV. Obtained results revealed that spraying tomato plants with (2.5mM) riboflavin five days before inoculation were reduced mosaic symptoms on treated plants to 25 % **Fig 4**. This result was in agreement with those of Zhang et al (2009), who reported the enhancement resistance of tobacco plant against *Alternaria alternata* and TMV after riboflavin treatment. Our results are also in accordance with those of Torkey (2016), who found a significant effect of 2mM riboflavin in reducing TMV symptoms on sweet pepper when applied just before viral infection. In addition, 2.5mM riboflavin treatment showed a significant suppressive influence on virus infectivity and virus concentration compare with other treatments **Figs 5 and 6**. This indicates that exogenous application of 2.5mM riboflavin before virus inoculation could enhance resistance against TMV in both L.L. host and host plants. These results are consistent with the findings of Torkey (2016) who observed the efficiency of low concentration of riboflavin and thiamin in reducing TMV infectivity and its concentration in *Capsicum annuum*. On the other hand, obtained results in time course experiment indicated that, disease severity and virus concentration were reduced and reached its minimum levels 6 to 8 days after treating with 2.5mM riboflavin **Figs 7 and 8**. Similar observations were also reported by Abdel- Monaim (2011) who observed the inhibition of disease severity of charcoal rot disease 5 to 7 days after riboflavin treating soybean plants.

Several studies have been explained how riboflavin can prime systemic plant defenses (Wang et al 2013; Polacios et al 2014). According to Darwish et al (2017), Riboflavin is necessary cofactor in diversing metabolic pathways that lead directly to promote plant growth and enabling higher resistance to pathogen attack. Obtained data denoted that, plant high and number of leaves were improved in each of non-inoculated and inoculated plant after treatment with 2.5mM riboflavin **Figs 1 and 2**. This assertion is also supported by the findings of Bondok and Thabet (2016) who reported that 1mM riboflavin foliar application enhanced tomato plant vigor and triggered plant defense TMV infection. Furthermore, numerous researchers demonstrated the role of riboflavin in priming disease defense reactions that include the expression of pathogenesis related (PR) proteins (Nie and Xu 2016). In present work, the transcription level of PR10 gene, as a molecular marker for SAR, was gradually increased in treated plants and peaked at 6 hours after foliar application of 2.5mM riboflavin **Fig 9**. Interestingly, the accumulation of CaPR-10, one of the members of PR10 protein, has displayed significantly phosphorylation, which enhanced resistance against TMV in *Capsicum annuum* as previously recorded (Ali et al 2018). Likewise, expression level of the PAL gene, a key enzyme of the phenylpropanoid pathway, was increased after riboflavin treatment at all-time points **Fig 9**. These results are in agreement with Taheri and Tarighi (2010) who explained the role of riboflavin to enhance plant defense responses through up regulation of *PAL* in rice- *R. solani* pathosystem. The literature obviously mentioned that riboflavin mediates many bioprocesses related with the increasing of peroxidation (Ji et al 2014). Our investigation cleared notable elevation of PO and PPO activities in both non-infected and TMV-infected plants at the 8^{th} day after treatment with 2.5mM riboflavin **Figs 10 and 11**. Same trend of results obtained by Abdel- Monaim (2011) who mentioned that treatment with either thiamine or riboflavin can induce elicitation of the PO and PPO activity in soya bean plants and reached maximum levels at 8 and 6 respectively. Bondok and Thabet (2016) also observed higher PO activity in riboflavin treated tomato plant at 7^{th} day after TMV inoculation. These results also are in line with Torky (2016) who observed enhancement in the activities of PO and PPO in *C. annuum* plants sprayed with thiamin and riboflavin providing resistance against TMV. Altogether, these results suggest that riboflavin might be involved in enhancing of plant defense responses.

5 Conclusion

In conclusion, this study provides an efficient approach that priming tomato defense responses against TMV infection. Foliar application with 2.5mM riboflavin before TMV challenge decreased disease severity and virus concentration, which associated with activation a series of defense responses including increasing in the expression of

defense-related genes (PR10 – and PAL genes) and the activities of antioxidant enzyme (PO and PPO).

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حث مقاومة نباتات الطماطم ضد فيروس تبرقش الدخان باستخدام الريبوفلافين

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فريدة محمد محمود* – أحمد محسن بندق – ولاء خليفة – فوزى أبو العباس

قسم أمراض النبات – كلية الزراعة – جامعة عين شمس – ص.ب 68 – حدائق شبرا 11241 – القاهرة – مصر

*Corresponding author: Faridamohamed@agr.asu.edu.eg

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تستحث المقاومة الجهازية للنبات باستخدام مواد طبيعية كوسيلة بديلة لمكافحة المرض. يستخدم الريبوفلافين (فيتامين ب٢) عادة كمحث غير حيوى لتحسين مقاومة النبات ضد ممرضات مختلفة. هذا العمل يهدف الى تقييم فعالية ثلاث تركيزات للريبوفلافين (0.5 & 2.5 و 5 ملى مول) لزيادة المقاومة ضد مرض التبرقش في نباتات الطماطم. وقد أظهرت نتائج الدراسة أن الأستعمال الخارجي لـ 2.5 ملى مول ريبوفلافين قبل 5 أيام من الحقن بالفيروس كان هو التركيز الأكثر فاعلية والذى أدى الى خفض نسبة وشدة الإصابة بنسبة 80% و75% على التوالي مقارنة بالنباتات الغير معاملة. وقد تم تأكيد النتائج المتحصل عليها سيروجيا بأستخدام أختبار الأليزا والذى أوضح أن 2.5 ملى مول ريبوفلافين خفض التركيز النسبى للفيروس بنسبة 46.4% فى النباتات المعاملة. كما تم تحديد التأثير على فاعلية الفيروس من خلال تكون البقع المحلية على العائل المشخص حيث انخفض عدد البقع على أوراق نباتات الـ *Nicotiana glutinosa* بالنسبة للكنترول. كما لوحظ أن المعاملة الخارجية بتركيز 2.5 ملى مول ريبوفلافين أظهرت تأثير ايجابى على نمو النباتات سواء في حالة النباتات المحقونة أو الغير محقونة بالفيروس حيث زاد معدل أطوال النباتات. وكذلك عدد الأوراق الى حوالى 30% و 78% على التوالي مقارنة بالكنترول المحقون. منحنى التثبيط حقق أن المعاملة بتركيز 5و2 ملى مول ريبوفلافين خفض أعراض الفيروس ووصلت لأعلى تأثير فعال من 6الى 9 أيام ثم انخفض بعد ذلك. بيانتنا قدمت أكثرمن ذلك حيث أن مستوى التعبير الجينى لجين الفينيل ألانين أمونيا لايز (*PAL*) و الـ *PR10* والتى تعتبر علامات على المقاومة الجهازية المكتسبة (SAR) أزدادت سريعا في نباتات الطماطم المعاملة والمحقونة خلال 1–3 أيام بعد المقاومة ;علاوة على ذلك فأن نتائج التحليل البيوكيميائية أظهرت ذلك حيث أن علامات الدفاع والتى تتضمن كلا من انزيم البيروكسيديز (POD) والبولى فينول اوكسيديز (PPO) زادت بعد أربعة أيام من المعاملة ووصلت للمستويات القصوى عند 8 أيام في النباتات المعاملة والمحقونة خلال فترة التجربة. وختاما، تحقق أن الريبوفلافين له تأثير شديد على الإصابة بفيروس الـ TMV كما تأكد ذلك من خلال خفض أعراض المرض مع حدوث التغيرات البيوكيميائية المرتبطة بتعظيم مقاومة النبات ضد الإصابة بفيروس ال TMV.