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ORIGINAL PAPER

Comparative Studies Between Treatments of Potato Tubers Slices with Three Amino Acids and Treatment with Salicylic Acid or Jasmonic Acid on the Infection by Dry Rot (*Fusarium solani*)

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ABSTRACT

The effect of treatment potato tuber slices with three different amino acids *i.e*., L-tyrosine, L-arginine or L-methionine was carried out to throw some light on their interaction with dry rot caused by *Fusarium solani* (Mart.) Sacc. L-methionine induced a large effect on disease severity; it caused a change in the interaction between host and pathogen from a state of susceptibility to a state of resistance. However, either salicylic acid or jasmonic acid led to a similar less effect. The obtained results overlooked the role of such hormones in disease resistance and led to postulate the occurrence of DNA methylation due to the treatment with L-methionine.

Keywords: Potato, *Solanum tuberosum*, Dry rot, *Fusarium solani*, L-tyrosine, L-arginine, L-methionine, Salicylic acid, Jasmonic acid.

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INTRODUCTION

Fusarium dry rot is one of the most important diseases of potato (*Solanum tuberosum* L.), affecting tubers in storage and seed pieces after planting and reduces crop establishment by killing developing potato sprouts (Wharton *et al*., 2006 and Aydin *et al.*, 2016). The different varieties of potato varied in their resistance to the disease and the nature of such variation among varieties in their susceptibility to infection by the pathogen still just speculations (Esfahani, 2005).

It is well established that synthetic chemicals such as salicylic acid, jasmonic acid and many other chemicals lead to induction of local and systemic resistance against pathogens in treated plants (Kuc, 2001; Sanz-Alferez *et al*., 2008; Klessig *et al*., 2018 and Tripathi *et al*., 2019).

Amino acids are the building blocks of proteins, they play an essential role in the regulation of growth and development in all living organisms. Recently, many researchers have reported that amino acids affect the plant immune systems against abiotic and biotic stresses (Kauffman *et al*., 2007; Cerdan *et al*., 2013; Kadotani *et al*., 2016; Rouphael and Colla 2018 and Rouphael *et al*., 2018).

A mixture of methionine and riboflavin (M+R) showed biocidal effect against several plant pathogens such as fungi and bacteria (*Botrytis cinerea*, *Peronophythora litchi*, *Verticillium* spp., *Pseudomonas syringae*, and *Agrobacterium tumefaciens*) under continuous illumination (2 h) depending on pH of the mixture (Tzeng and DeVay, 1989).

The disease severity of strawberry powdery mildew was reduced by foliar application with mixture of methionine (1mM) and riboflavin (26.6 μM). The efficacy of disease reduction was enhanced by foliar spray with the mixture (M+R) in combination with metal ions copper, iron and surfactants of sodium dodecyl sulfate, Triton X-100, Tween-20, or oxyalkylene methyl siloxane (Silwet L-77) (Wang and Tzeng, 1998).

Pre-harvest treatment of tomato fruits with 0.5, 1, and 5 mM L-arginine decreased lesion size caused by *Botrytis cinerea* on fruits, and increased the activities of phenylalanine ammonia-lyase (PAL), polyphenoloxidase (PPO), chitinase (CHI), and β-1,3-glucanase (GLU), compared with untreated fruits with L-arginine (Zheng *et al*., 2011).

Foliar application of L-phenylalanine and ferulic acid to pea plants significantly reduced conidial germination of *Erysiphe pisi* on pea leaves. Treatment with different concentrations (50, 100 and 150 mg/L) of L-phenylalanine increased the activity of PAL, while ferulic acid increased activity of this enzyme at the higher concentrations (100 and 150 mg/L) in pea leaves compared to untreated plants (Bahadur *et al.*, 2012).

The present investigation was carried out to study the effect of application of L-tyrosine, L-arginine or L-methionine to potato tuber slices on their ability to reduce infection with *Fusarium solani* in comparison to treatment by salicylic acid or jasmonic acid.

MATERIALS AND METHODS

1. Fungal isolate:

Fusarium solani (Mart.) Sacc was previously isolated from potato tubers infected with dry rot (Mohamed and Mostafa, 2020) and identified according to Booth (1971). Also, its ability to cause severe rot on potato tuber slices was proved (Mohamed and Mostafa, 2020). The fungus was sub-cultured on potato sucrose agar medium (PSA). A sever isolate was chosen for the foregoing study. A conidia suspension in sterilized distilled water was prepared from 10 days old cultures, and conidia density was adjusted before use to be 10^6 conidia /mL.

2. Source of Potato tubers:

Potato tubers of cv. Balmoral were obtained from the Institute of Vegetable Research, Agricultural Research Centre, Giza, Egypt. The tubers were stored at least for one month at room temperature (22±2°C).

3. Compounds tested:

L-tyrosine, L-arginine and L-methionine in two concentrations at 2.5 or 5.0 mM were tested. Salicylic acid and Jasmonic acid were tested at 5.0 mM. Moreover, mixtures of 5mM of either salicylic acid + methionine or jasmonic acid + methionine were also tested for their effect on potato tuber slices and *Fusarium solani*. Non-treated slices were used as a control.

4. Effect of treatment potato tuber slices with amino acids in comparison with salicylic acid or jasmonic acid on dry rot severity and No. of conidia on the infected area:

Tubers were washed several times under tap water, and then surface sterilized using 0.5% NaOCl for five minutes thereafter, washing in sterilized distilled water. Surface sterilized tubers were cut into 1 cm-thick slices with the exclusion of the top and the basal parts. Slices were dipped in the desired solution for two hours. Slices were transferred to Petri dishes (15 cm in diameter) containing two wetted filter papers. Five slices were put in every dish and three dishes as replicates were used for each particular treatment. Inoculation of slices was carried out one hour after transferring in dishes by spreading gently 0.5 mL of *F. solani* spore suspension over every slice. Dishes were incubated at 24±1°C in the dark for 3 days.

5. Disease assessment:

Three days after potato slices inoculation, each slice was taken for photograph. The percentage of the infected tuber tissue surface was assessed according to Sharawy (1988) using the following scale: 0%: no infection occurred, 12.5 %: 1/8 of the slice surface was infected, 25 %: 1/4 of the slice surface was infected, 50%: half of the slice surface was infected and 100%: all slice surface was completely infected. As for determination the number of conidia on the infected slices, the upper inoculated surface was removed then ground in 10 mL distilled water. The number of conidiospores was counted using haemocytometer slide on a light microscope. The disease was expressed as a number of conidia/ cm^2 of inoculated surface, as well as the estimation of the percentage of slice surface area covered by fungal mycelia (Mostafa *et al.*, 2018).

6. Effect of Potato Sucrose Agar (PSA) medium amended with amino acids on the growth and sporulation of *F. solani*:

Sterilized melted Potato Sucrose Agar (PSA) medium was amended with two concentrations, *i.e.*, 2.5 or 5.0 mM of each amino acid, L-tyrosine, L-arginine or L-methionine and then poured in Petri dishes. After solidification, a disk (5mm in diameter) bearing fungal growth of *F. solani* was transferred from the active margin of fungal growth culture to the center of every dish, treated with amino acids and untreated ones. All dishes were incubated at 24±1°C for 3 days, then the diameter of growth (cm) and the number of conidia produced on each cm^2 fungal growth were estimated (Lopez-Berges *et al.*, 2010).

7. Statistical analysis:

All the previously described experiments were carried out three times. Data were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS) (Littell *et al.*, 1996). The mean values were compared using Duncan's multiple range test at $P \leq 0.01$.

RESULTS

1. Effect of treatment potato tuber slices with amino acids on dry rot severity incited by *F. solani* and No. of conidia on the infected area:

Results illustrated in Figs. (1 and 2) indicate that tyrosine at 2.5 or 5.0 mM showed no effect in reducing the infected area of potato slices. Such result was clear with methionine (2.5 mM). However, L-methionine (5mM) has significantly reduced the infected area followed by L-arginine (5mM). Data illustrated in Fig. (3) show that all treatments with amino acids (except tyrosine 2.5mM) significantly reduced fungal sporulation on the inoculated slices. Tyrosine at 2.5 mM treatment showed a similar effect to control untreated slices. However, L-methionine at 5.0 mM was the highest effective treatment in reducing fungal sporulation.

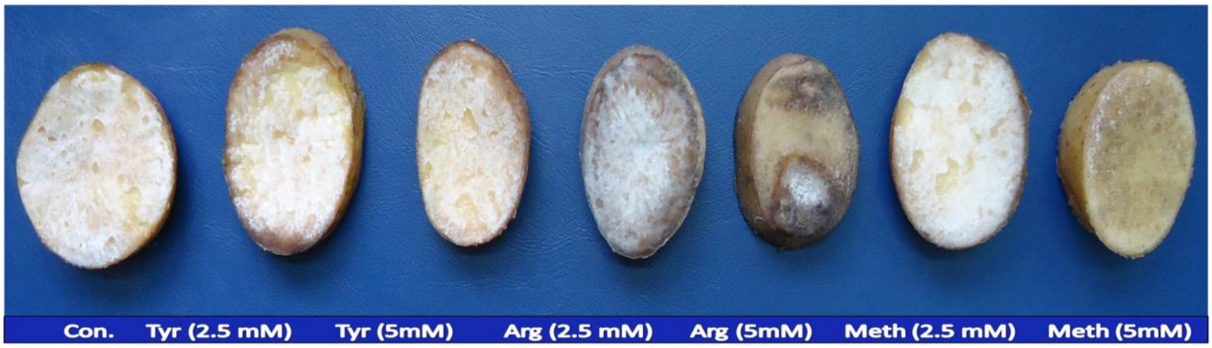

Fig. (1): Effect of treatment potato slices with different amino acids on severity of dry rot caused by *F. solani* (3 days after inoculation), (Con: control, Tyr: tyrosine, Arg: L-arginine, Meth: L-methionine).

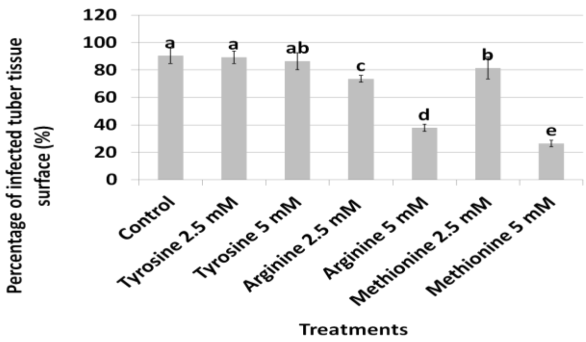

Fig. (2): Effect of treatment potato slices with different amino acids on the percentage of infected tuber tissue surface of dry rot caused by *F. solani*. The same letters are meaning no significant differences between two bars according to Duncan's multiple range test at P≤0.01.

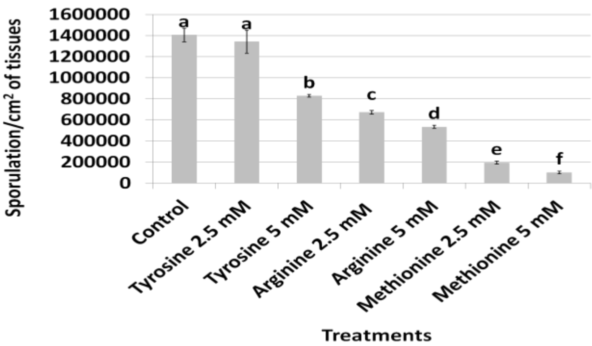

Fig. (3): Effect of treatment potato slices with different amino acids on sporulation of *F. solani* the causal of dry rot on potato tuber slices. The same letters are meaning no significant differences between two bars according to Duncan's multiple range test at P≤0.01.

2. Effect of Potato Sucrose Agar (PSA) medium amended with amino acids on the growth and sporulation of *F. solani*:

Experiments concerning the tested amino acids were expanded to study their effect on *F. solani* growth and sporulation on potato sucrose agar medium amended by L-tyrosine, L-arginine and L-methionine at two different concentrations (2.5 or 5.0 mM). Data are presented in Figs. (4 and 5). It is clear from data in Fig. (4) that all tested amino acids showed no inhibitory effect towards the fungal growth except methionine at 5 mM that showed a significant decrease in fungal growth. As for fungal sporulation on PSA treated with amino acids, No. of conidia/ cm^2 was significantly reduced by all treatments except L-arginine at 2.5 mM.

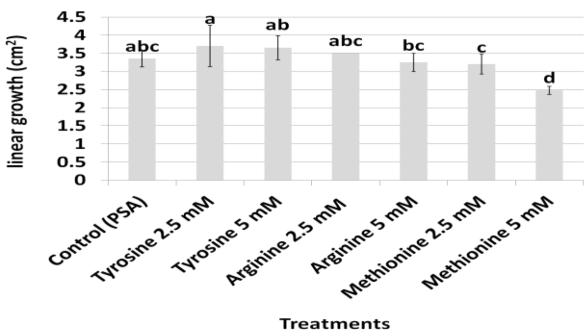

Fig. (4): Effect of PSA treated with different amino acids on the linear growth of *F. solani* after 3 days incubation at 24±1ºC. The same letters are meaning no significant differences between two bars according to Duncan's multiple range test at P≤0.01.

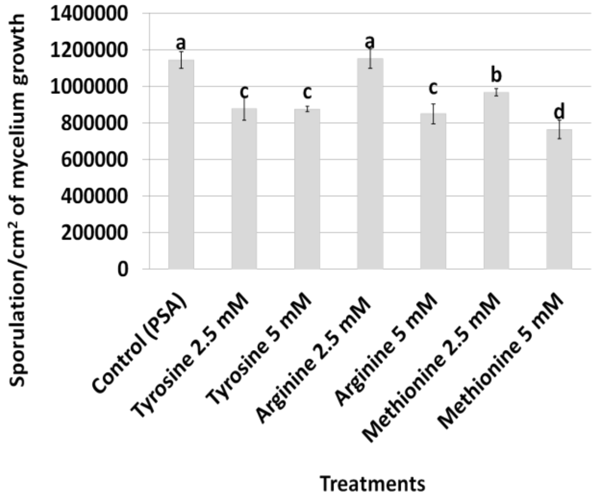

Fig. (5): Effect of PSA treated with different amino acids on sporulation of *F. solani* after 3 days incubation at 24±1°C. The same letters are meaning no significant differences between two bars according to Duncan's multiple range test at P≤0.01.

3. Effect of treatment potato tuber slices with 5 mM of both salicylic acid and jasmonic acid on potato tuber reaction to infection with dry rot caused by *F. solani*:

It is clearly shown from results illustrated in Figs (6 and 7) that each of salicylic acid and jasmonic acid caused little effect on the percentage of infected tuber tissue surface with mycelial growth of *F. solani* in comparison to control but any of them did not reach the effect of L-methionine. As for fungal sporulation, it was obvious that both compounds, salicylic acid and jasmonic acid significantly reduced fungal sporulation on treated slices. Treatment with jasmonic acid was the highest effective treatment in reducing fungal sporulation (Fig. 8).

Fig. (6): Effect of potato slices treatment with 5mM of jasmonic acid or salicylic acid on disease severity of dry rot caused by *F. solani* at 3 days after inoculation.

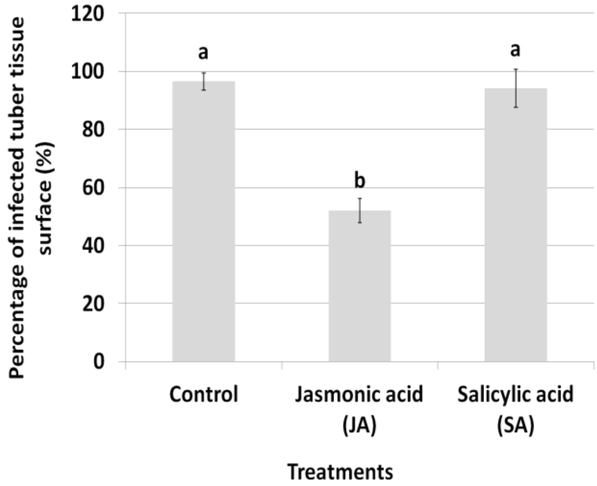

Fig. (7): Effect of treatment potato slices with 5 mM of both jasmonic acid and salicylic acid on the percentage of dry rot caused by *F. solani*. The same letters are meaning no significant differences between two bars according to Duncan's multiple range test at P≤0.01.

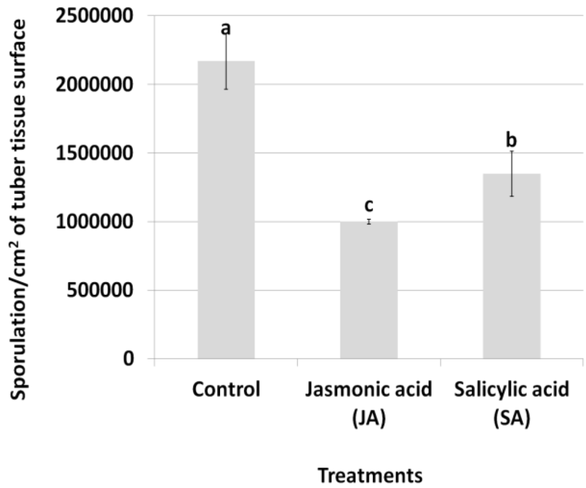

Fig. (8): Effect of treatment potato slices with 5 mM of both jasmonic acid and salicylic acid on sporulation of *F. solani* the causal of dry rot. The same letters are meaning no significant differences between two bars according to Duncan's multiple range test at P≤0.01.

L-methionine was mixed either by salicylic acid or jasmonic acid to test the effect of the mixture on the percentage of infected tuber tissue surface. The results of this study are presented in Figs. (9, 10 and 11). It is clearly shown from these results that L-methionine when mixed with jasmonic acid led to significant decrease of both the percentage of infected tuber tissue surface and sporulation in comparison with salicylic acid + L-methionine.

Fig. (9): Effect of the combination with L-methionine and jasmonic acid or salicylic acid on disease severity of dry rot caused by *F. solani* after 3 days of inoculation.

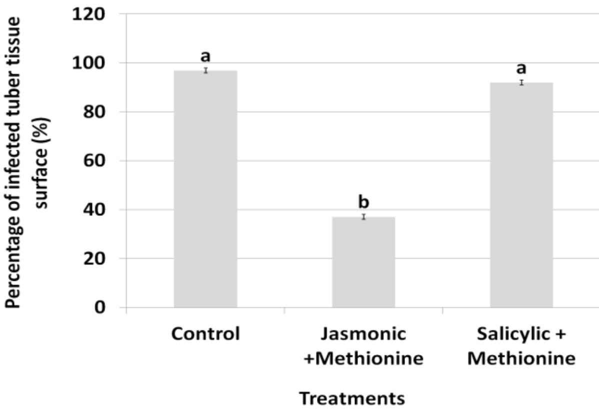

Fig. (10): Effect of the combination of methionine and each of jasmonic acid or salicylic acid on the percentage of infection of tuber tissue surface caused by *F. solani*. The same letters are meaning no significant differences between two bars according to Duncan's multiple range test at P≤0.01.

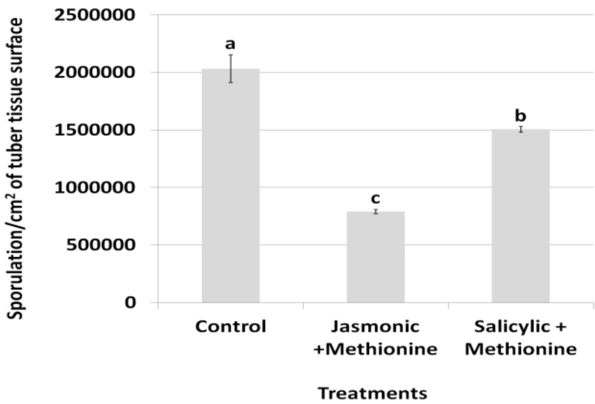

Fig. (11): Effect of the combination of methionine and each of jasmonic acid or salicylic acid on sporulation of *F. solani* the causal of dry rot disease. The same letters are meaning no significant differences between two bars according to Duncan's multiple range test at P≤0.01.

DISCUSSION

It is well established that the result of interaction between potato tuber tissue and fusarium tuber dry rot pathogen is determined by the degree of resistance of potato variety to infection. The nature of such resistance is still just speculations and needs further investigations.

Plant signals which mediate plant disease resistance were intensively studied in necrotrophic and biotrophic pathogens. In the first case, jasmonic acid or its derivatives are triggers mediating plant resistance to diseases. However, plant resistance to biotrophs greatly depends upon salicylic acid or its derivatives (McDowell and Dangl, 2000; Kunkel and Brooks, 2002; Glazebrook, 2005; Verhagen *et al.*, 2006; Guerreiro *et al.*, 2016; Brouwer *et al.*, 2020 and Gomi, 2020).

In the present work, interesting results were obtained by L-methionine treatment, where it induced a change in reaction of potato tubers to fusarium dry rot from the susceptible to the resistance case. Such results of disease incidence on potato slices well corresponded with the effect of methionine treatment on both the linear growth of mycelium and sporulation of the pathogen on semi synthetic medium. In the same respect, methionine significantly decreased the severity (size of cankers) of bacterial canker on lime (*Citrus aurantifolia*) caused by *Xanthomonas citri* subsp. *citri* (Hasabi *et al.*, 2014). The authors stated that reduction of lime canker severity was correlated with induced plant resistance by enhancing the expression of PR-2gene (β-1,3-glucanase) and also activating antioxidant enzymes, *i.e.*, peroxidase, phenylalanine ammonia-lyase (PAL) and catalase.

How the amino acid L-methionine causes this action, to investigate such reason potato tuber slices were treated with salicylic acid or jasmonic acid then inoculated with *F. solani*. Data obtained indicated that both compounds caused a little effect on disease severity. These results led to the postulate that disease resistance did not occur via salicylic acid or jasmonic acid and led to the purpose the presence of other types of resistance due to the treatment with L-methionine.

Recently, epigenetic was postulated as a new area of plant disease resistance. Such epigenetic is going through methylation of DNA in the presence of L-methionine as a donor of methyl group (Mao *et al.*, 2015 and Deleris *et al.*, 2016). These findings may explain how L-methionine induces disease resistance in plants.

Deleris *et al*. (2016) summarized recently biological relevance with DNA methylation and demethylation in plant immunity to non-viral pathogens. They discussed the relevance of epigenetic regulatory processes in the transcriptional and co-transcriptional control of immune-responsive genes and their role in antimicrobial immune responses.

AUTHOR CONTRIBUTIONS

Mohamed, M.H. conceived and designed the experiments; collected the isolates and performed the experiments. Mohamed, M.H. and Mostafa, M.H. carried out the data analysis, discussed the study and wrote the article. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST:

The authors declare no conflict of interest exists.

REFERENCES

Aydin, M.H.; Pala, F. and Kaplan, C. 2016. Potato tuber sprout rot caused by *Fusarium sambucinum* in Turkey. Scientific Papers. Series A. Agronomy, Vol. LIX, 189-193.

Bahadur, A.; Singh, D.P.; Sarma, B.K. and Singh, U.P. 2012. Foliar application of L-phenylalanine and ferulic acids to pea plants: induced phenylalanine ammonia-lyase activity and resistance against *Erysiphe pisi*. Archives of Phytopathology and Plant Protection, 45(4): 398-403.

Booth, C. 1971. The genus Fusarium. Commonwealth Mycological Institute, CAB International. Kew, Surrey, England, 237pp.

Brouwer, S.M.; Odilbekov, F.; Burra, D.D.; Lenman, M.; Hedley, P.E.; Grenville-Briggs, L.; Alexandersson, E.; Liljeroth, E. and Andreasson, E. 2020. Intact salicylic acid signaling is required for potato defense against the necrotrophic fungus *Alternaria solani*. Plant Molecular Biology, 104: 1-19.

Cerdan, M.; Sanchez-Sanchez, A.; Jorda, J.D.; Juarez, M. and Sanchez-Andreu, J. 2013. Effect of commercial amino acids on iron nutrition of tomato plants grown under lime-induced iron deficiency. J. Plant Nutr. Soil Sci., 176: 859-866.

Deleris, A.; Halter, T. and Navarro, L. 2016. DNA methylation and demethylation in plant immunity. Annu. Rev. Phytopathol., 54: 579-603.

Esfahani, M.N. 2005. Susceptibility assessment of potato cultivars to Fusarium dry rot species. Potato Research, 48: 215-226.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol., 43: 205-27.

Gomi, K. 2020. Jasmonic acid: an essential plant hormone. Int. J. Mol. Sci., 21: 1261.

Guerreiro, A.; Figueiredo, J.; Silva, M.S. and Figueiredo, A. 2016. Linking jasmonic acid to grapevine resistance against the biotrophic oomycete *Plasmopara viticola.* Front. Plant Sci., 7: 565.

Hasabi, V.; Askari, H.; Alavi, S.M. and Zamanizadeh, H. 2014. Effect of amino acid application on induced resistance against citrus canker disease in lime plants. Journal of Plant Protection Research, 54 (2): 144-149.

Kadotani, N.; Akagi, A.; Takatsuji, H.; Miwa, T. and Igarashi, D. 2016. Exogenous proteinogenic amino acids induce systemic resistance in rice. BMC Plant Biology, 16: 60.

Kauffman, G.L.; Kneivel, D.P. and Watschke, T.L. 2007. Effects of a biostimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol production of perennial ryegrass. Crop Science, 47(1): 261-267.

Klessig, D.F.; Choi, H.W. and Dempsey, D.A. 2018. Systemic acquired resistance and salicylic acid: past, present, and future. Molecular Plant-Microbe Interactions, 31(9): 871-888.

Kuc, J. 2001. Concepts and direction of induced systemic resistance in plants and its application. Eur. J. Plant Pathol., 107(1):7-12.

Kunkel. B.N. and Brooks, D.M. 2002. Cross talk between signaling pathways in pathogen defense. Current Opinion in Plant Biology, 5: 325-331.

Littell, R.C.; Milliken, G. A.; Stroup, W. W.; Wolfinger, R. D. and Cary, N. C. 1996. SAS system for mixed models. SAS Institute Inc., 633pp.

López-Berges, M.S.; Rispail, N.; Prados-Rosales, R.C. and Pietro, A.D. 2010. A nitrogen response pathway regulates virulence functions in *Fusarium oxysporum* via the protein kinase TOR and the bZIP protein MeaB. The Plant Cell, 22: 2459-2475.

Mao. D.; Yu, F.; Li, J.; Van de Poel, B.; Tan, D.; Li, J.; Liu, Y.; Li, X.; Dong, M. and Chen, L. 2015. FERONIA receptor kinase interacts with S adenosylmethionine synthetase and suppresses S-adenosylmethionine production and ethylene biosynthesis in Arabidopsis. Plant Cell Environ, 38: 2566-2574.

McDowell, J.M. and Dangl, J.L. 2000. Signal transduction in the plant immune response. Trends Biochem. Sci., 25: 79-82.

Mohamed, M.H. and Mostafa, H.M. 2020. Isolation and identification of *Fusarium* spp. associate with potato tuber dry rot disease: growth rate and pathogenicity in relation to elicitation of phytoalexins in Inoculated tissues. World Journal of Pharmaceutical and Life Sciences, 6(4): 27-37.

Mostafa, H.M. 2018. Elicitation of phytoalexin rishitin in potato tuber slices infected by *Fusarium* spp. does it consider a factor of pathogenicity. Int. J. Phytopathol. 07(02): 53-61.

Rouphael, Y. and Colla, G. 2018. Synergistic biostimulatory action: designing the next generation of plant biostimulants for sustainable agriculture. Front. Plant Sci., 9:1655.

Rouphael, Y.; Spíchal, L.; Panzarová, K.; Casa, R. and Colla, G. 2018. High-throughput plant phenotyping for developing novel biostimulants: from lab to field or from field to lab? Front. Plant Sci., 9: 1197.

Sanz-Alferez, S.; Mateos, B.; Alvarade, R. and Sanchez, M. 2008. Induction in tomato plants is not effective against root-knot nematode infection. Eur. J. Plant Pathol., 120:417-425.

Sharawy, N.M. 1988. The Effect of Gamma Radiation on Potato Tuber Rot in Egypt., Ph.D. Thesis. Fac. Agric., Ain Shams Univ., 204 pp.

Tripathi, D.; Raikhy, G. and Kumar, D. 2019. Chemical elicitors of systemic acquired resistance-salicylic acid and its functional analogs. Current Plant Biology, 17: 48-59.

Tzeng, D.D. and DeVay, J.E. 1989. Biocidal activity of mixtures of methionine and riboflavin against plant pathogenic fungi and bacteria and possible modes of action. Mycologia, 81(3): 404-412.

Verhagen, B.W.M.; Van Loon, L.C. and Pieterse, C.M.J. 2006. Induced resistance signaling in plants. Floriculture, Ornamental and Plant Biotechnology, Global Science Books, Volume III: 334-343.

Wang, S.Y. and Tzeng, D.D. 1998. Methionine-riboflavin mixtures with surfactants and metal ions reduce powdery mildew infection in strawberry plants. J. Amer. Soc. Hort. Sci., 123(6): 987-991.

Wharton P.S., Tumbalam P., Kirk W.W., 2006. First report of potato tuber sprout rot caused by *Fusarium sambucinum* in Michigan. Plant disease, 90: 1460-1460.

Zheng, Y.; Sheng, J.; Zhao, R.; Zhang, J.; Lv, S.; Liu, L. and Shen, L. 2011. Preharvest L-arginine treatment induced postharvest disease resistance to *Botrytis cinerea* in tomato fruits. J. Agric. Food Chem., 59: 6543-6549.