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Research Article

Induction of resistance in tomato against early blight

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ABSTRACT

Tomato is used both in fresh and semi cooked form, therefore, pesticides residues neither accepted nor allowed by stakeholders. Plant resistance inducers (PRIs) or elicitors have shown promising potential to be an effective but safe alternate of pesticides. In this study, we investigated the role of different PRIs against *Alternaria solani*, causal agent of early blight of tomato. PRIs used in this study were 0.1 mM methyl Jasmonate (MeJA), 1.0 mM salicylic acid (SA), 1mg/ml chitosan (CH). In addition, 0.2 mM sodium nitroprusside (SNP) and 1mg/pot calcium carbide (CaC₂) were also used as donors of nitric oxide (NO) and ethylene (ET) respectively. *A. solani* was isolated from infected tomato samples which were collected from suburbs of Rawalpindi and Islamabad. Pathogenicity was confirmed by Koch's postulates. Post 48 hrs of priming with PRIs, tomato seedlings were then sprayed by *A. solani* inoculum. Plants treated with distil water were considered as mock treatment whereas challenged with pathogen as a positive control. Disease symptoms recorded 10 days post inoculation (dpi). MeJA treatment gave the best result as we got percentage of EB index (PEBI) 14.15% compared to positive control (44.98%). Plants treated with CaC₂ which is donor of ET and CH gave statistically non-significant results compared to MeJA while gave significant results compared to SA and positive control. Overall, resistant inducers delayed the disease and make the plants tolerant rather immune against the pathogen. Our data imply that there is potential of activating innate immunity in tomato against one of most destructive and widely distributed pathogens by the application of PRIs.

Keywords: Tomato; *Alternaria solani*; methyl Jasmonate; calcium carbide; Chitosan.



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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) along eggplant, pepper and potato, is a member of solanaceae family. Tomato is thought to be originated in Peru and later was introduced in rest of world by traders and invaders. Currently, there is hardly any cuisine in the world which does not include tomato as an ingredient. Thus, there is surge in tomato cultivation and now tomato is considered as second most cultivated vegetable after potato. Tomato is considered a healthy food for being high in secondary metabolites such as lycopene, flavonoids, phytosterols, and polyphenols. Pakistan ranks 34th in global tomato production, with annual production of 572837 tons (FAOSTAT 2017). Sustainable tomato production in Pakistan is confronting many barriers which are both biotic and abiotic in nature. Major bacterial diseases of tomato reported from Pakistan includes wilt (*Ralstonia solanacearum*) (Aslam et al., 2017), canker (*Clavibacter michiganensis*) (Bibi et al., 2018), leaf spot (*Xanthomonas vesicatoria*) (Ahmad et al., 2023), leaf speck (*Pseudomonas syringae* pv tomato), and fungal diseases like damping off (*Rhizoctonia solani*) (Gondal et al., 2019), late blight (*Phytophthora infestans*) and early blight (*Alternaria solani*) (Ansari et al., 2023).

Early blight (EB) of tomato caused by *Alternaria solani* (Ellis & Martin) Sorauer is one of the most devastating tomato diseases which has worldwide distribution. *A. solani* belongs to the order hyphales of class Deuteromycetes (fungi imperfecti) (Agrios, 2005). Pathogens can harm all above-ground parts of the plant, including leaf, stem, and fruit, at any stage of growth. EB disease occurs frequently at relative humidity (55-61%) and temperature (14-36 °C) (Riaz et al., 2021). Annual economic yield losses owing to EB are estimated upto 79% (Adhikari et al., 2017) during disease conducive conditions. However, pathogens also cause significant crop losses in semi-arid areas where there is frequent night-time dew occurs. There are reports on prevalence and management of early blight in tomato crop growing in different parts of Pakistan (Chohan et al., 2015; Saleem et al., 2016; Raza et al., 2016). Overall, plantations in Pakistan are becoming more vulnerable against pathogens such as *Alternaria* spp. due to climate change. Pakistan is considered as 7th most vulnerable country to climate change which may result in surge of plant diseases (Raja et al., 2018).

EB can be managed by cultural practices to remove infected plants residue as pathogen over winters in it. Crop rotation for 3-4 years removes EB pathogen. However, in intensified agriculture it seems not a practical option. Most of the tomato breeding lines are either susceptible to moderately resistant against EB (Vloutoglou, 1999; Gardner and Shoemaker, 1999). Therefore, use of moderate resistant varieties (Mountain Pride, Supreme, Gold, Fresh and Belle) and fungicides such as Mancozeb, copper oxychloride, chlorothalonil and carbendazim are widely used to eliminate EB (Mate et al., 2005; Patil et al., 2002). But due to development of resistance in EB against fungicides and deleterious effects of fungicides on environment and human health, fungicides application does not seem a safe option (Reshu and Khan, 2012). Alternatively, biocontrol agents (BCAs) such as *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus pumilis* are also used against EB (Yazici et al., 2011; Zghair et al., 2014). Though, BCAs are considerably safe option but not without limitations. There are limitations include fluctuations in environment, dosage optimization as well uniform application of BCAs etc.

Therefore, researchers are looking an option to activate the innate immunity of plants without causing any environmental and health hazards. In contrast to animal immunity which based on antigen-antibody interaction, plant immunity relies heavily on production of different signaling molecules. There is bulk of published material on the role of signaling molecules such as salicylic acid (SA), methyl jasmonates (MeJA), ethylene (ET), nitric oxide (NO) in plant defence (Spoel et al., 2003; Hong et al., 2008). Though, cross talk of these signaling molecules is complex but are considered as potential to fend off majority pathological ingresses. Plant resistance may be local such as hypersensitive response (HR) or systemic depending upon the signaling molecules. There are two types of systemic resistance in plants; one is systemic acquired resistance (SAR) while other is induced systemic resistance (ISR). SA is marker of SAR whereas MeJA and ET are markers of ISR. In addition, detection of indispensable part of pathogens such as flagella (bacteria) and chitin (fungi) also help plant to remain resistance against majority of pathogens in its life span. Such indispensable parts are called pathogen associated molecular patterns (PAMPs) and immunity triggered by the detection of PAMPs is known as PAMP triggered immunity (PTI) (Spoel and Dong, 2012; Alexandersson et al., 2016). Other, key signalling molecule produced when plants are challenged by pathogens is nitric oxide (NO) which reacts with hydrogen peroxide to confine pathogen through a response commonly known as HR (Hong et al., 2008; Yu et al., 2014). Therefore, researchers adopted a proactive approach to exogenously treat plants with signalling molecules such as salicylic acid (Agamy et al., 2013), chitosan (Sathiyabama et al., 2014), sodium nitroprusside (Lai et al., 2011), methyl jasmonate (Kepczynska and Krol, 2012) and calcium carbide (Siddiq et al., 2012) to activate innate resistance. PRIs primed host cells are exhibiting stronger and lifetime resistance against subsequent pathogen attacks (Goellner and Conrath, 2008). It is worth to mention that this kind of management strategy has more potential in vegetable production as these commodities are mostly consumed as fresh or in semi-cooked form, therefore, residual effects of fungicide neither permissible nor acceptable by stake holders (Murgia et al., 2004; da-Rocha and Hammerschmidt, 2005; Pastor et al., 2013). In present study, we sought to find the impact of exogenous application of PRIs against EB of tomato.

MATERIALS AND METHODS

The research work was conducted in the Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi. Tomato variety Rio Grande and line 95017 were grown in cups containing mixture of decomposed coco dust, cow dung, sand, leaf litter and soil in a ratio (2:1:1:1:1) respectively.

Collection and Isolation of Plant Pathogenic Fungi

The tomato leaves showing peculiar disease symptoms of early blight (EB) were collected from experimental fields and tomato growing areas of Islamabad and Rawalpindi. Plant samples were preserved in paper bags and stored at 4°C for the isolation of pathogens. The infected plant tissues along with healthy tissues were cut into small pieces (5-10 mm) and were surface sterilized by dipping into 1% clorox (sodium hypochlorite) for 2 minutes to kill surface contaminants. Four pieces were placed on petri plate containing potato dextrose agar (PDA) and Czapek-Dox Agar (CDA) to initiate fungal growth at 25±1 °C for seven days. Pure culture was prepared by hyphal tip method.

Pathogenicity Assay and Confirmation of Koch's Postulates

Pathogenicity test was carried out on cultivars, Reograndi and 95017. Ten days old culture of *A. solani* obtained from EB infected tomato leaves was used to make conidial suspension (5×10^6 conidia/mL) in sterilized distilled water. Six-week-old tomato plants were inoculated by using hand run sprayers and all of the test plants were kept in polythene chamber at 20°C and 95% relative humidity for 48 hours (Stammler *et al.*, 2014). Pathogen re-isolated and grown on culture media to confirmed Koch's postulates.

Induction of Resistance and Application of Fungal Inoculum

Experiment was carried out according to completely randomized design (CRD) with five replications and five plants per replication were used. Six weeks old tomato line-95017 was used as experimental planting material for resistance induction experiment. Different plant resistant inducers viz., 1.0 mM salicylic acid (SA), 0.1 mM methyl jasmonate (MeJA), 1mg/ml chitosan (CH) were used while 1mg/pot calcium carbide (CaC_2) and 0.2 mM sodium nitroprusside (SNP) were used as donors of ethylene (Et) and nitric oxide (NO) respectively (Faheed *et al.*, 2005; Mahmood *et al.*, 2010; Kepczynska and Krol, 2012; Sathiyabama *et al.*, 2014; Noorbakhsh and Taheri, 2016). Tomato plants primed with inducers were then challenged with pathogen after 48 hours of post induction of resistance. The experiment was carried out two times with five seedlings for each variety and all the experimental plants were observed regularly. Disease development data was taken ten days of post inoculation (dpi). EB severity on each leaf of the sprayed plants was recorded on a scale of 0 to 5, where 0= no visible lesions on leaf, 1= up to 10% leaf area was affected, 2=11%-25%, 3=26%-50%, 4=51%-75% and 5=>75% leaf area affected or leaf abscised (Vakalounakis, 1983). Symptoms produced by the pathogen were observed 10 dpi. The plants were rated resistant if scored 0-1, moderately resistant if scored 2-3 and susceptible if scored 4-5. The disease scales were converted into percentage of EB index (PEBI) for each plant using the following formula (Pandey *et al.*, 2003). Plants sprayed with water considered as mock treatment whereas treated with pathogen considered as a positive control.

$$\text{PEBI} = \frac{\text{Sum of all ratings}}{\text{No. of leaves sampled} \times \text{Maximum Disease Scale}} \times 100$$

RESULTS AND DISCUSSION

Isolation and Characterization of Pathogen Associated with Infected Tomato Plants

A. solani causal organism of EB showed black and circular growth on PDA while on CDA dark brown growth appeared after 7 dpi at 25 °C (Figure:1 a). Microscopic studies showed that conidiophores of *A. solani* were single or in small groups, straight or flexuous and the colour was brown to dusky yellowish. Conidia were mostly single rather short or elongate typically with both cross and longitudinal septa. The conidia were also with different shapes such as straight or ovoid acuminate with long apical beak having brown or pale golden color. The mycelium of *A. solani* grew with circular margin and smooth surfaced colony (Figure 1 b).

Pathogenicity Assay on Tomato Plants

Our pathogenicity assay not only confirmed Koch's postulates but also revealed that tomato cultivar I95017 was more susceptible compared to Reograndi. Thus, line-95017 was further used in resistance induction experiment (Figure 2 a,b).

Effect of Inducers in Tomato Plants Against EB

Exogenously treated tomato plants with PRIs such as MeJA, SA, CH and CaC_2 and SNP as donor of ET and NO were later inoculated with EB spores by foliar spray method. Plants treated with *A. solani* inoculum only showed symptoms after 5-6 dpi whereas incubation period of treated plants extended upto 10 days (Figure 3). It is imperative to mention that disease severity (blight spots/leaf) on treated leaves was reduced with the passage of time. In stark contrast, lesions increased in size on untreated plants as time passed. Disease severity data which recorded both for untreated and treated plants after 10 dpi showed that MeJA treatment had best result as we got PEBI 14.15% compared to

positive control (44.98%). PEBI value 14.15% fall in moderately resistant category while 44.98% fall in susceptible category (Figure 4). However, plants treated with inducers CaC₂ which is donor of ET and CH also gave statistically non-significant results compared to MeJA while gave significant results compared to SA and positive control. Though, plants treated with SNP which is donor of NO showed closeness with CH but it also statistically non-significant with CH but significant with rest of treatments such as MeJA, CaC₂ and SA and positive control. Though, SA treatment improved resistance against pathogen but failed to bring significant level as PEBI value 30.13% fall in rating scale 3 which means plants were still susceptible against subsequent attack by pathogen. In hindsight, resistance in tomato plants prior to treatment with signaling molecules such as MeJA, CaC₂, SNP and CH overall improved but not able to bring plants to resistant level rather making them tolerant against pathogen. However, SA treatment seemed no potential in improvement of tolerance level and plants even after treatment with SA remained susceptible to EB (Figure 4).

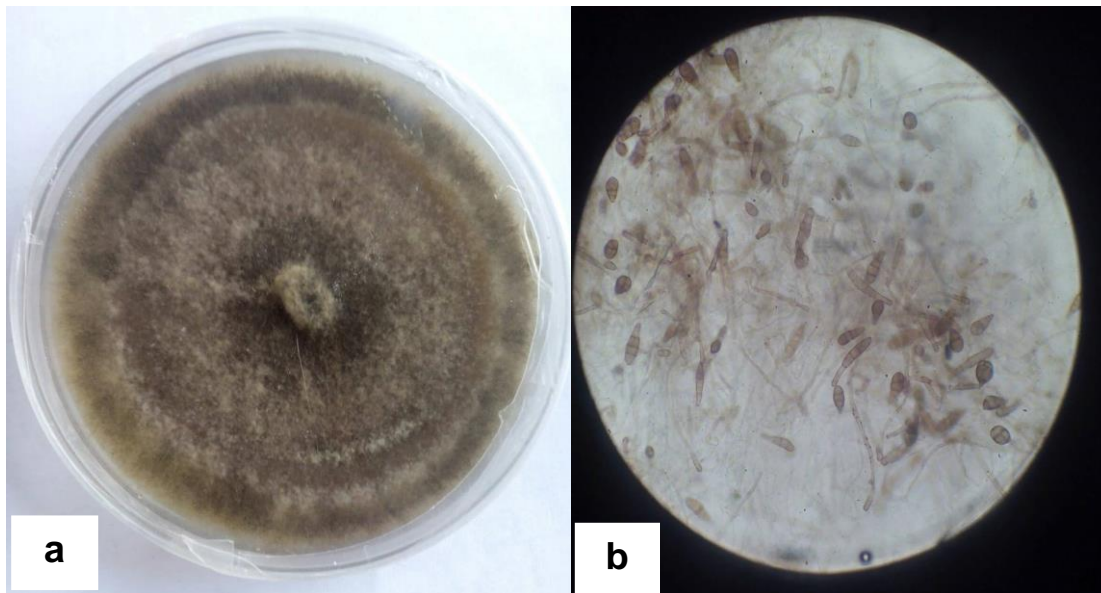


Figure 1. *Alternaria solani* growing on CDA media. (a) Dark brown concentric *A. solani* culture of CDA plate. (b) *A. solani* cross-sectional and beaked conidiophores.

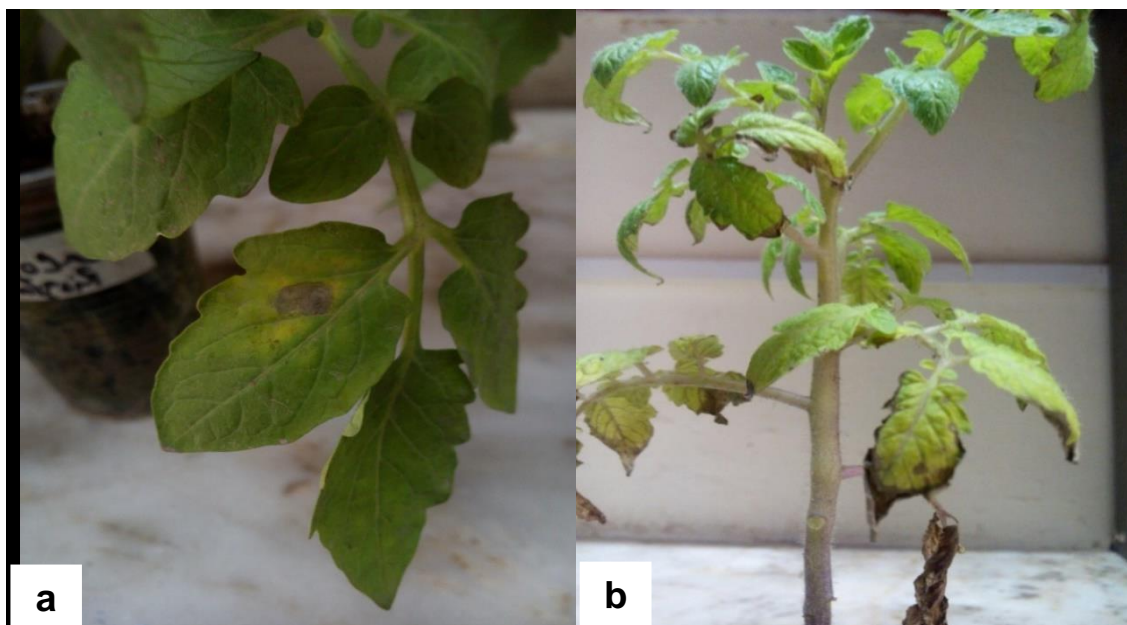


Figure 2. Pathogenicity assay on tomato plants after 10 days of post inoculation with EB (a) symptoms of EB on Riogrande (b) Symptoms on line-95017.

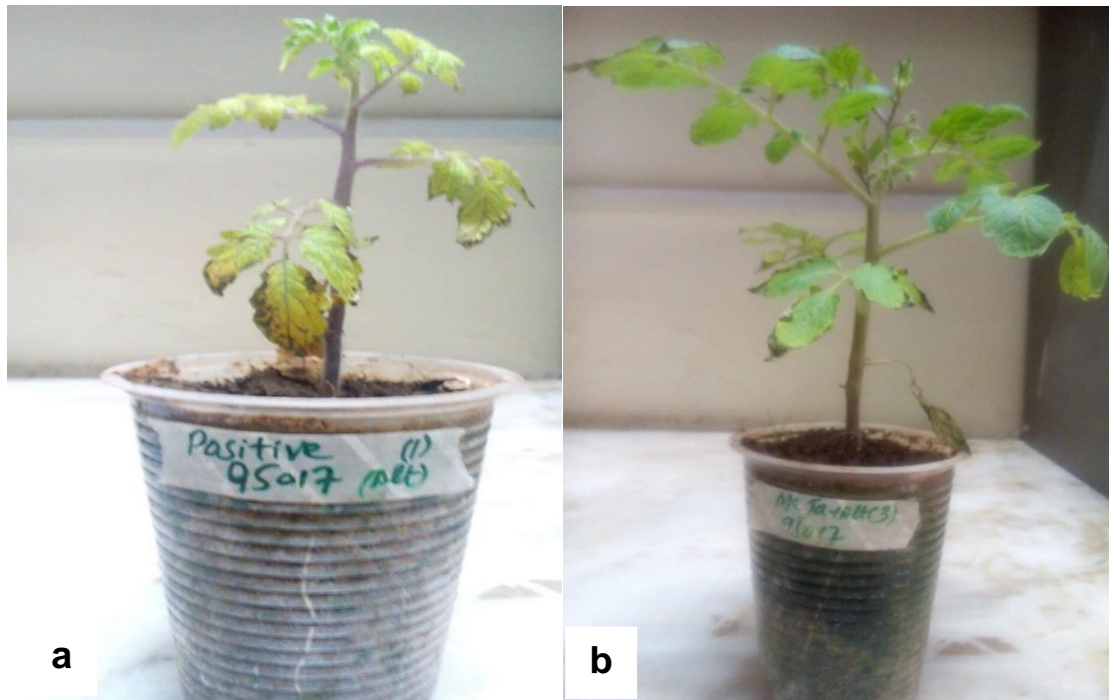


Figure 3. Early blight symptoms shown by inducer treated and un-treated plants after 10 days of *A. solani* inoculation (a) Inducer Un-treated Plant (b) Inducer Treated Plant.

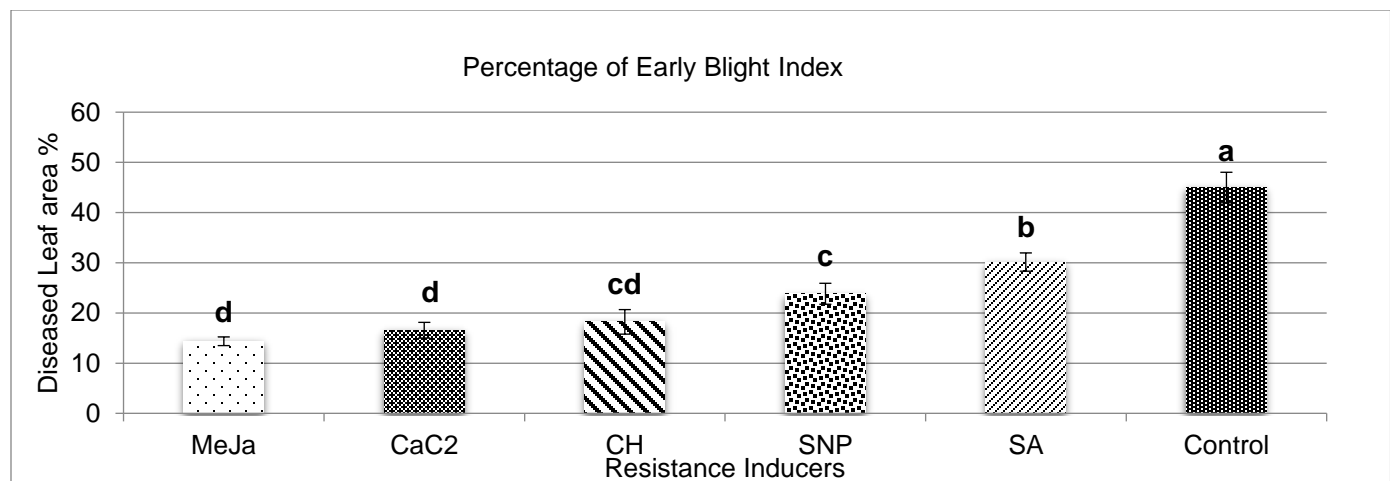


Figure 4. Treatments in column which share similar letter with each other are statistically non-significant but those treatments which don't share same letter with each other are statistically significant $p \leq 0.05$.

Current study was planned to observe the impact of exogenous application of PRIs on susceptible tomato cultivar 95017 when challenged by *A. solani*. Our data suggested that MeJA had reduced susceptibility 3 times compared to positive control. After exogenous application of MeJA, there is drastic shift from susceptibility to moderately tolerant plant. Similarly, exogenous application of ET and CH also gave good results compared to SNP, SA and positive control. Our results re-affirmed past findings on model plants such as *Arabidopsis thaliana* where researchers found that plant induce different type of systemic resistance when attacked by necrotrophs, hemi-biotrophs or biotrophs. Against necrotroph which killed host cells and later colonize it; induced systemic resistance (ISR) act as a bulwark whose markers are MeJA and ET. Glazebrook (2005) reviewed that *A. solani* is a necrotroph fungus, therefore, tomato plants which were exogenously sprayed with MeJA or drenched with CaC_2 (donor of ET) gave best results while exogenous application of SA was found not much effective. MeJA or ET worked synergistically with each other while antagonistically with SA. Exogenous application of SA reduced susceptibility compared to positive control plants could be due to the activation of *NPR1* which is master regulator of both ISR and systemic acquired resistance (SAR) (Pieterse, 2004; Van et al., 2008). Similarly, exogenous application of CH had reduced disease because CH reception

by host plant elicits resistance mechanism. It is plausible to mention that enhanced resistance by CH application could be compared to MeJA and ET application. Chitosan application has close physical proximity with chitin and its application is perceived by plant as fungal attack and resulted in activation of PTI. Chitosan also showed antimicrobial properties and is widely used in agriculture to mitigate various pre- and post- harvest pathogens (Padamini et al., 2024). It is pertinent to mention that exogenous application of SNP (donor of NO) was not as effective as compared to MeJA or ET but still more effective than SA. It is reported that NO when react with reactive oxygen species (ROS), hydrogen peroxide, it may lead to HR. However, ROS production is more associated with biotrophs or hemi-biotrophs rather necrotrophs, therefore, NO is not much effective as compared to other inducers (Hong et al., 2008; Yu et al., 2014). Our results re-affirmed that inducers could prime host cells against subsequent attack by pathogens. Disease is rule rather than exception in plants as pathogens have array of dispersal, penetration and colonization mechanisms. Despite, efforts of decades there is no fool proof system to completely dislodge these mechanisms (Panno et al., 2021). Biological control involving resistant genes and BCAs provided some relief but due to tripartite interaction, it could lead to unpredicted results. Additionally, environmental factors, mode of application, microbiome composition also shape the outcome of BCAs effectiveness. Climate change, trade and travel, mutations resulted into virulent strains of pathohgens which can overcome innate plant immunity or BCAs. Though, chemicals provide a kind of readymade solution but not without ecological and health hazards. In this background, inducers which are environmentally safe, long lasting and not impaired by environmental fluctuations seem to be the most reliable option, if applied prior to pathogen attack (Lahlali et al., 2022; Singh et al., 2020). Induce resistance is an alternate complementary approach which showed promise and needed to be further explored at broad scale.

CONCLUSION

From our study, PRIs have the potential to mitigate pathogens but success neither overwhelming nor general. Rather, it depends on pathogen mode of nutrition and corresponding signaling molecule. Moreover, PRIs help the plants to become tolerant but not immune as numerous factors such as plant genetics, growth stage, growth conditions, mode and dose of application etc. also play pivotal role. This research paves a way for the future use of PRIs under control conditions.

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REFERENCES

- Adhikari, P., Oh, Y. & Panthee, D. R. (2017). Current status of early blight resistance in tomato: an update. *International Journal of Molecular Sciences*, 18(10),2019.
- Agamy, R., Alamri, S., Moustafa, M.F. & Hashem, M. (2013). Management of tomato leaf spot caused by *Alternaria tenuissima* Wiltshire using salicylic acid and agrileen. *International Journal of Agriculture and Biology*, 15(2): 266-272.
- Agrios, G. N. 2005. Plant Pathology, 5th.ed. Academic Press, Inc. New York. 803.
- Ahmad, Zohaib., Alfehaid,M.A., Saira Asghar,S., Manan, A., Gill,S. & Memoona Bibi,M. (2023). Efficient Diagnosis of Bacterial Leaf Spot in Tomato Plants using Deep Learning CNN Models. *Plant Bulletin*, 2(2), 93-103.
- Alexandersson, E., Mulugeta, T., Lankinen, Å., Liljeroth, E. & Andreasson, E. (2016). Plant Resistance Inducers against Pathogens in Solanaceae Species-From Molecular Mechanisms to Field Application. *International Journal of Molecular Sciences*, 17(10),1673.
- Ansari., M., Ahmed., S., Abbasi, A., Hamad, N. A., Ali, H. M., Khan, M. T., Haq, I.U. & Zaman, Q. U. (2023). Green synthesized silver nanoparticles: a novel approach for the enhanced growth and yield of tomato against early blight disease. *Microorganisms*, 11(4), 886.
- Aslam, M. N., Mukhtar, T., Hussain, M. A., & Raheel, M. (2017). Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. *Journal of Plant Diseases and Protection*, 124, 585-590.
- Bibi, A., Ahmad, M., & Hussain, S. (2018). Prevalence of (*Clavibacter michiganensis* subsp. *michiganensis*) causal organism of bacterial canker in weed species in tomato fields of North West Pakistan. *Sarhad Journal of Agriculture*, 34(1), 123-129.
- Chohan, S., Perveen, R., Mehmood, M.A., Naz, S. & Akram, N. (2015). Morpho-physiological studies, management and screening of tomato germplasm against *Alternaria solani*, the causal agent of tomato early blight. *International Journal of Agriculture and Biology*, 17,111-118.
- da-Rocha, A.B. & Hammerschmidt, R. (2005). History and perspectives on the use of disease resistance inducers in horticultural crops. *Hort. Technology*, 15(3), 518-529.

- FAO.2017. Food and Agriculture Organization of the United Nations. *FAOSTAT: Crops and livestock products - Tomato production*.
- Faheed, F. A., Abd-Elaah, G.A. & Mayzen, A. (2005). Alleviation of disease effect on tomato plants by heat shock and salicylic acid infected with *Alternaria solani*. *International Journal of Agriculture and Biology*, 5, 783-789.
- Gardner, R.G. & Shoemaker, P.B. (1999). 'Mountain Supreme' early blight-resistant hybrid tomato and its parents, NC EBR-3 and NC EBR-4. *Horticultural Science*, 34, 745-746.
- Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*, 43, 205-27.
- Gondal, A. S., Rauf, A., & Naz, F. (2019). Anastomosis Groups of *Rhizoctonia solani* associated with tomato foot rot in Pothohar Region of Pakistan. *Scientific reports*, 9(1), 3910.
- Goellner, K. & Conrath, U. (2008). Priming: it's all the world to induced disease resistance. *European Journal of Plant Pathology*, 121, 233-242.
- Hong, J.K., Yun, B.W., Kang, J.G., Raja, M.U., Kwon, E.J., Sorhagen, S., Chu, C., Wang, Y.Q. & Loake, G.J. (2008). Nitric oxide function and signaling in plant disease resistance. *Journal of Experimental Botany*, 59, 147-154
- Kepczynska, E. & Krol, P. (2012). The phytohormone methyl jasmonate as an activator of induced resistance against the necrotroph *Alternaria porri* f. sp. *solani* in tomato plants. *Journal of Plant Interactions*, 7(4), 307-315.
- Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z. & Barka, E. A. (2022). Biological control of plant pathogens: A global perspective. *Microorganisms*, 10(3), 596.
- Lai, T., Wang, Y., Li, B., Qin, G. & Tian, S. (2011). Defense responses to tomato fruit to exogenous nitric oxide during postharvest storage. *Postharvest Biology and Technology*, 62, 127-132.
- Mahmood, R., Yaseen, M., Arshad, M. and Tanvir, A. (2010). Comparative effect of different calcium carbide based formulations on growth and yield of wheat. *Soil and Environment*, 29(1), 33-37.
- Mate, G.D., Deshmukh, V.V., Jiotode, D.J., Chore, N.S. & Dikkar, M. (2005). Efficacy of plant products and fungicides on tomato early blight caused by *Alternaria solani*. *Research on Crops*, 6(2), 3490-351.
- Murgia, I., Tarantino, D., Vannini, C., Bracale, M., Carravieri, S. & Soave, C. (2004). *Arabidopsis thaliana* plants overexpressing thylakoidal ascorbate peroxidase show increased resistance to Paraquat-induced photooxidative stress and to nitric oxide-induced cell death. *Plant Journal*, 38(6), 940-953.
- Noorbakhsh, Z. & Taheri, P. (2016). Nitric oxide: a signaling molecule which activates cell wall-associated defense of tomato against *Rhizoctonia solani*. *European Journal of Plant Pathology*, 144(3), 551-568.
- Padamini, R., Bhumita, P., Hazarika, S., NM, R., Gowda, G. R. V., Chaturvedi, K., Panigrahi, C. K., & Karanwal, R. (2024). Exploring the Role of Chitosan: A Natural Solution for Plant Disease and Insect Management. *Archives of Current Research International*, 24(6), 102-116.
- Pandey, K.K., Pandey, P.K., Kallo, G. & Banerjee, M.K. (2003). Resistance to early blight of tomato with respect to various parameters of disease epidemics. *Journal of General Plant Pathology*, 69, 364-371.
- Panno, S., Davino, S., Caruso, A. G., Bertacca, S., Crnogorac, A., Mandić, A., ... & Matić, S. (2021). A review of the most common and economically important diseases that undermine the cultivation of tomato crop in the mediterranean basin. *Agronomy*, 11(11), 2188.
- Pastor, V., Luna, E., Mauch-Mani, B., Ton, J. & Flors, V. (2013). Primed plants do not Forget. *Environmental and Experimental Botany*, 94, 46-56.
- Patil, M.J., Ukeyand, S.P., Raut, B.T. (2002). Evaluation of Fungicides and Botanicals for the Management of Early Blight (*Alternaria solani*) of Tomato. *PKV Research Journal*, 25(1), 49-51.
- Pieterse, C. M. & Van Loon, L.C. (2004). NPR1: the spider in the web of induced resistance signaling pathways. *Current Opinion Plant Biology*, 7(4), 456-464.
- Raja, M. U., Mukhtar, T., Shaheen, F.A., Bodlah, I., Jamal, A., Fatima, B. Ismail, M. & Shah, I. (2018). Climate change and its impact on plant health: A Pakistan's prospective. *Plant Protection*, 02 (02), 51-56.
- Raza, W., Ghazanfar, M.U., Yasir, I., Ahmed, K.S., Haider, N. & Rasheed, M.H. (2016). Management of early blight of tomato through the use of plant extracts. *International Journal of Zoology Studies*, (1), 01-04.
- Reshu, M. & Khan, M.M. (2012). Role of different microbial origin bioactive antifungal compounds against *Alternaria spp.* causing leaf blight of mustard. *Journal of Plant Pathology*, 11(1), 1-9.
- Riaz, H. M., Chohan, S., & Abid, M. (2021). Occurrence of tomato early blight disease and associated *Alternaria* species in Punjab, Pakistan. *Journal of Animal & Plant Sciences*, 31(5).
- Saleem, M.Y., Akhtar, K.P., Iqbal, Q., Asghar, M., Hameed, A. & Shoaib, M. (2016). Development of tomato hybrids with multiple disease tolerance. *Pakistan Journal of Botany*, 48(2), 771-778.
- Sathiyabama, M., Akila, G. & Charles, R.E. (2014). Chitosan-induced defence responses in tomato plants against early blight disease caused by *Alternaria solani* (Ellis and Martin) Sorauer, *Archives of Phytopathology and Plant Protection*, (47)14, 1777-1787.
- Siddiq, S., Yaseen, M., Arshad, M. & Ahmed, N. (2012). Effect of calcium carbide on photosynthetic characteristics, growth and yield of tomato cultivars. *Pakistan Journal of Agricultural Sciences*, 49(4), 505-510.

- Singh, S., Kumar, V., Dhanjal, D. S., & Singh, J. (2020). Biological control agents: diversity, ecological significances, and biotechnological applications. *Natural Bioactive Products in Sustainable Agriculture*, 31-44.
- Spoel, S.H., Koornneef, A., Claessen, S.M., Korzelius, J.P., Van-Pelt, J.A., Mueller, M.J., Buchala A.J., Metraux, J.P., Brown, R., Kazan, K. & Van-Loon, L. C. (2003). NPR1 modulates cross-talk between salicylate-and jasmonate-dependent defense pathways through a novel function in the cytosol. *The Plant Cell*, 15(3), 760-770.
- Spoel, S.H. & Dong, X. (2012). How do plants achieve immunity? Defence without immune cells. *Nature Reviews Immunology*, 12, 89-100.
- Stammler, G., Franziska, B., Jasmin, P., Simone, M. & Vanessa, T. (2014). Pathogenicity of *Alternaria*-species on potatoes and tomatoes. In Fourteenth Euro Blight Workshop PPO-Special Report, 16, 85-96.
- Vakalounakis, D.J. (1983). Evaluation of tomato cultivars for resistance to *Alternaria* blight. *Annals of Applied Biology*, 102, 138-139.
- Van, S. C., S. Van der Ent & Pieterse, C. M. (2008). Plant immune responses triggered by beneficial microbes. *Current Opinion in Plant Biology*, 11(4),443-448.
- Vloutoglou, I. (1999). Evaluation of tomato cultivars and hybrids for resistance to *Alternaria solani* infection. *Tests of Agrochemicals and Cultivars*, 20,48-49.
- Yazici, S., Yanar, Y. & Karaman, I. (2011). Evaluation of bacteria for biological control of early blight disease of tomato. *African Journal of Biotechnology*, 10(9) 1573-1577.
- Yu, M., Lamattina, L., Spoel, S.H. & Loake, G.J. (2014). Nitric oxide function in plant biology: a redox cue in deconvolution. *New Phytologist*, 202(4), 1142-1156.
- Zghair, Q.N., Lal, A.A., Mane, M.M. & Simon, S. (2014). Effect of bio-agents and fungicide against early blight disease of tomato (*Lycopersicon esculentum* L.). *International Journal of Plant Protection*. 7(2), 330-333.