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Foliar Application of Chitosan and Yeast Elicitor Facilitate Reducing Incidence and Severity of Alternaria Leaf Blight of Tomato and Brinjal

F. H. Tumpa and M. A. R. Khokon*

Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

***Corresponding author:** Khokon MAR, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, E-mail: atiq.ppath@bau.edu.bd

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Abstract

Two pot experiments were conducted in the net house to investigate whether aqueous solution of chitosan and yeast elicitor can suppress Alternaria leaf blight of tomato and brinjal. Aqueous solutions of chitosan and yeast elicitor were applied on seeds and on the foliage of tomato and brinjal at three growth stages following 0.02, 0.05, 0.1 and 0.2% concentration respectively. The chitosan and yeast elicitor-treated plants were further inoculated artificially to create sufficient disease pressure to monitor incidence and severity. Differential responses were recorded for chitosan and yeast elicitor considering both growth stages and type of crops. Chitosan performed superior in tomato plants while yeast elicitor in brinjal plants considering both disease incidence and severity. In both cases, 42 DAT was more suitable for chitosan and yeast elicitor spray to get maximum disease suppression. Chitosan and yeast elicitor at 0.2% showed superior performance in reducing blight incidence and severity by Alternaria.

Keywords: Chitosan; Yeast elicitor; *Alternaria solani*; Tomato; Brinjal

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Introduction

Tomato (*Lycopersicon esculentum Mill.*) and Brinjal (*Solanum melongena L.*), are widespread and popular vegetables crops grown all places in Bangladesh and around the world. At present, the average production is 413610 MT and 310354 MT per hectare for tomato and brinjal [1]. in Bangladesh. The productions of these two vegetables are far below as compared to the major brinjal and tomato growing countries of the World. The reasons behind the low yields of these vegetables are diseases, insects and poor management practices.

Tomato and brinjal crops are vulnerable to a number of fungal diseases. Among the fungal diseases, *Alternaria* leaf blight caused by *Alternaria solani* is the most important one, causing damping off of seedling stage, blight on the foliage and stem canker at later growth stage and fruit decay on mature fruits [2,3]. The pathogen is seed-borne in nature rendering the disease more difficult to manage. As a result, seed treatments by various fungicides are very common practice in the country leading residual effects, environmental pollution and enormously increasing the risk of fungicide resistance. Hence, an alternative approach needs to work out with organic compounds to combat the disease.

Various compounds viz. salicylic acid, β-aminobutyric acid, chitosan and 2, 6 dichloroisonicotinic acids are known to elicit resistance in crop plants [4-10]. reported that pre-harvest applications of MeJA or chitosan had a long-lasting effect on the reduction of *B. cinerea* incidence during postharvest as well as an enhancer effect on the induction of PR and PGIP gene expression. Chitosan is a naturally occurring constituents found in the fungal cell wall. Chitosan produced by the fungus in the host-parasite interaction both enters the plant cells and accumulates within the fungal cell. The action of chitosan in initiating phytoalexin production, protecting pea tissue from *F.solani f. sp. pisi*, and/or directly terminating fungal growth indicates it may have a central role in disease resistance [11,12]. reported that chitosan was effective in protecting pearl millet plants against downy mildew under both greenhouse and field conditions by inducing resistance against the pathogen. Thus, chitosan formulation can be recommended for seed treatment in the management of downy mildew disease. Similarly, the capacity of inducing resistance

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against viral diseases by chitosan is known since years. Initially, it was shown that treatment of bean (*Phaseolus vulgaris*) leaves with the polymer decreased the number of local necrotic lesions caused by Alfalfa Mosaic vVrus (AMV) by triggering SAR [13-15]. Yeast cell-wall Extracts (YE) had high phytoalexin elicitor activation soybeans and induced resistance against barley powdery mildew in the normally highly susceptible cv. Golden Promise. Following treatment with YE there was rapid stimulation of Phenylalanine Ammonia-Lyase (PAL) activity and faster formation of papillae in response to attempted fungal penetration. We also examined the role of YE in ROS and NO production in cellular level which might have involvement in inducing resistance [16]. Yeast derived elicitor reduced the severity of infections caused by *Botryis cineria* and *Rhizoctonia solani* on lettuce [17]. It reduced the infection up to 90% in the glass house condition and *Rhizoctonia solani* infections were reduced up to 50%-70% induced resistance against *Penicillium digitatum* in grape by *Candida oleophola* [18]. When yeast-cellulose formulations applied to the leaf of kiwifruit significantly suppressed the liberations of conidia of *Botrytis cinerea* [19]. Therefore, resistance elicitor's *viz.* chitosan and yeast elicitor solution was used to study their effect on controlling *Alternaria* leaf blight disease and inducing resistance in comparison to untreated tomato and brinjal plants. The objectives of this research is to identify, isolate and inoculate the fungal pathogen *Alternaria solani* in tomato and brinjal plants and to know the bio-efficacy of chitosan and yeast elicitor for controlling *Alternaria* leaf blight disease.

Material and Methods

The experiments were conducted in the Laboratory of Biosignaling, Bioactive Compounds and Bio formulation, Department of Plant Pathology and Seed Pathology Centre (SPC), Bangladesh Agricultural University, Mymensigh-2202. Tomato and brinjal seeds were collected from the farmers of Mymensingh districts. These seeds were stored in zip-lock bags in refrigerator for further studies.

Preparation of chitosan solution

To prepare 0.3% stock solution of chitosan, 3 g dried chitosan was slowly dissolved in glacial acetic acid following continuously stirring by magnetic stirrer then diluted by distilled water to a volume of 1000 mL which was equivalent to 0.3%. From the stock solution 0.02%, 0.05%, 0.1% and 0.2% chitosan solution was prepared by adding required amount of distilled water.

Preparation of yeast elicitor solution

To prepare 0.3% Yeast Elicitor solution, Yeast (*Saccharomyces cerevisiae*) was first cultured in YEPDA broth (Yeast extract 1%, Peptone 2% and Dextrose 2%) in 250 mL Erlenmeyer flask having all the ingredients and then incubated on an orbital platform shaker at 300°C and 140 rpm for 72 hrs. After 72 hrs of incubation the broth was filtrated and filtrates was collected and subsequently mixed with ethanol solution following the key outlines by Cakir, et al. [20], which was equivalent to 0.3%. From the stock solution 0.02%, 0.05%, 0.1% and 0.2% yeast elicitor solution was prepared by adding required amount of distilled water.

Isolation, identification and purification of pathogenic fungi

Diseased leaves showing typical symptoms of *Alternaria* leaf blight were collected from the fields. To isolate the fungal pathogens, leaves were washed under running tap water and cut into small pieces followed by surface sterilization by 10% NaOCl solution for 30 seconds. The sterilized leaves were then washed several times by distilled water and blotted between sterile filter papers. The sterilized pieces were transferred into Petri dishes contained Potato Dextrose Agar (PDA) for incubation. For purification several sub-culture were performed on PDA medium following single spore culture method and kept at 20 ± 2°C for further studies. Morphological characters of the isolate were studied on PDA culture [21]. Fungal isolates were kept on PDA at 20 ± 2°C for 7 days. To induce sporulation, cultures were transferred on 23-25°C for 6 days on PDA at natural day light with 16 h/day light. Conidial suspensions were prepared [22]. Spore density was counted by a haemocytometer and adjusted to 105 conidia per mL.

Experimental set up under *in vivo* condition

According to the experimental design, the seedlings were incubated at 21, 42 and 63 DAT which are reported as the most vulnerable stages of foliar infection for tomato plants after transplanting. During inoculation, the seedlings were sprayed with either 0.1% yeast elicitor solution or 0.1% chitosan solution consecutively for 3 days to induce resistance against *Alternaria solani*. Since after inoculation, the pathogen suspension was subsequently sprayed to assess the disease incidence and severity.

Assessment of disease incidence and disease severity

Percent disease incidence was estimated according to the following formula given by James [23]:

Percent disease incidence=(Number of diseased leaves/Total number of leaves) × 100

To record disease severity, percentage of leaf area with necrotic spots and proportion of chlorosis were assessed separately for all unfolded leaves and arithmetic means for single plants were calculated.

Percentage of each foliar disease severity was recorded as following equation:

Percent disease severity=(Number of infected leaves per category × category number)/(Total leaves examined × 100)

Data on the disease severity was recorded after every ten days intervals from flowering stage to onwards using 0-5 disease rating scale as shown in the **Table 1** according to Boedo, et al. [22]

Table 1: Disease rating scale for the assessment of Alternaria blight of tomato and brinjal.

Scale	Disease incidence	Description
0	0	Leaves free from leaf spot
1	0%-5%	0-5 percent leaf area infected and covered by spot, no spots on petiole and branches
2	6%-20%	6-20 percent leaf area infected and covered by spot, some spots on petiole
3	21%-40%	21-40 percent leaf area infected and covered by spot, spots also seen on petiole, branches
4	41%-70%	41-70 percent leaf area infected and covered by spot, spots also seen on petiole, branches, stem
5	>70%	>71 percent leaf area infected and covered by spot, spots also seen on petiole, branch, stem, fruits

Analysis of data

The obtained data were analyzed by analysis of variance ANOVA, the mean values were compared using the Duncan's multiple test range test at 0.05 probability level. The analysis was done using MSTAT C package program.

Results

Disease incidence and severity of *Alternaria* blight of tomato and brinjal at 21 DAT

Alternaria blight of tomato was assessed at 21 DAT under inoculated condition in net-house experiments. In this treatment, a dose-dependent and statistically different incidence and severity were observed for chitosan and yeast elicitor treatment. All the concentration of chitosan and yeast elicitor showed the suppression of disease incidence and severity in tomato plants. Chitosan @ 0.2% showed the least disease incidence and severity followed by 0.2% yeast elicitor which were lower than positive control (Rovral @ 0.2%) **(Figure 1)**.

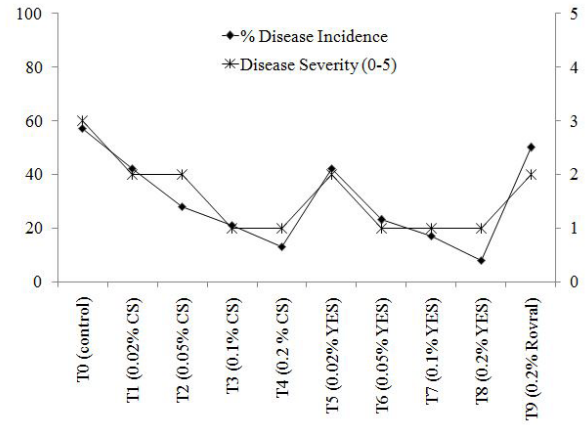

Figure 1: Incidence and severity of Alternaria blight of tomato at 21 DAT.

On the other hand, brinjal plants also showed a dose-dependent suppression of incidence and severity of *Alternaria* blight for both chitosan and yeast elicitor. Intriguingly the suppression was more evident in yeast elicitor treatment compared to chitosan. Although 0.1 and 0.2% chitosan showed significant suppression, but 0.1 and 0.2% yeast elicitor solution also showed superior suppression of incidence and severity of *Alternaria* blight of brinjal under inoculated condition **(Figure 2)**.

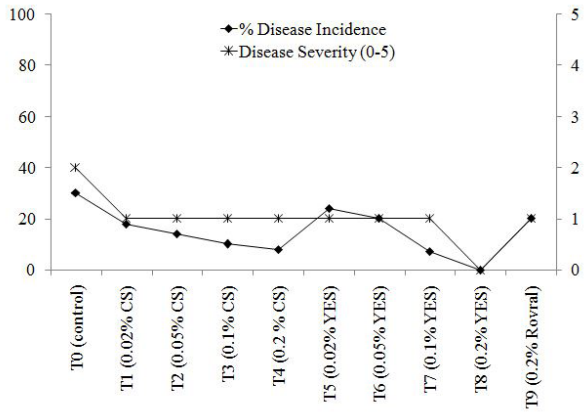

Figure 2: Incidence and severity of Alternaria blight of brinjal at 21 DAT.

Disease incidence and severity of *Alternaria* blight of tomato and brinjal at 42 DAT

Chitosan and yeast elicitor were applied on the foliage of tomato and brinjal plants at 42 DAT followed by artificial inoculation by *Alternaria solani* to assess disease incidence and severity **(Figures 3 and 4)**. In case of tomato, least incidence was recorded in 0.1% and 0.2% yeast elicitor and chitosan solution. Among all the treatments yeast elicitor @ 0.2% was most effective to suppress the disease incidence and severity.

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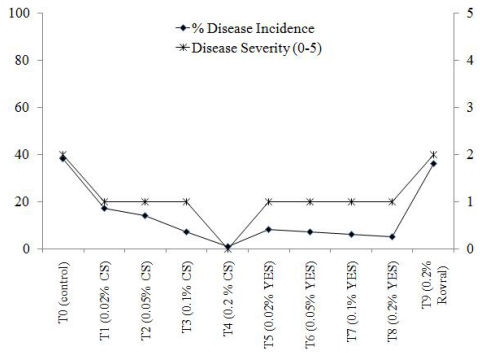

Figure 3: Incidence and severity of Alternaria blight of tomato at 42 DAT.

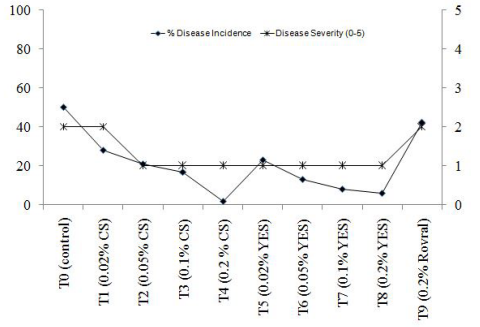

Figure 5: Incidence and severity of Alternaria blight of tomato at 63 DAT.

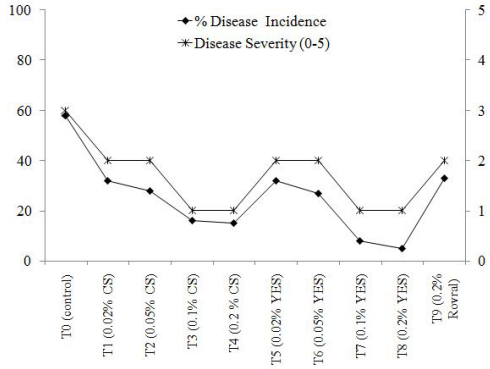

Figure 4: Incidence and severity of Alternaria blight of brinjal at 42 DAT.

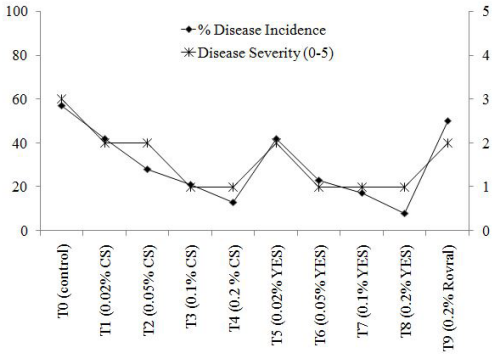

Figure 6: Incidence and severity of Alternaria blight of brinjal at 63 DAT.

In brinjal plants, disease incidence and severity showed a dose-dependent trend. Both incidence and severity was least in 0.2% chitosan. On the other hand, all concentrations of yeast elicitor showed superior performances compared to positive control Rovral @ 0.2% **(Figure 4)**. Both tomato and brinjal plants showed superior performances by chitosan and yeast elicitor solution compared to chemical control. Disease incidence and severity was almost absent in some cases treated by either 0.2% chitosan or 0.2% yeast elicitor solution.

Disease incidence and severity of Alternaria blight of tomato and brinjal at 63 DAT

Chitosan and yeast elicitor were applied on the foliage of tomato and brinjal plants at 63 DAT followed by artificial inoculation by *Alternaria solani* to assess the disease incidence and severity. Both yeast elicitor and chitosan showed a dose-dependent response to suppress disease incidence and severity **(Figures 5 and 6)**. Chitosan @ 0.2% showed superior response giving least disease incidence, while chitosan @ 0.2% also showed promising disease suppression compared positive control (Rovral @ 0.2%). Although there was distinct variation in disease incidence, but disease severity was almost similar in all treatments compared to positive control.

In brinjal plants, chitosan and yeast elicitor showed similar response like tomato plants at 63 DAT. Both chitosan and yeast elicitor @ 0.2% showed best performances compared to other treatments and positive control. The effect of yeast elicitor was more evident in brinjal plants than chitosan and chemical treatment **(Figure 6)**.

Discussion

The experiments were designed to observe the effect of foliar application of aqueous solution of chitosan and yeast elicitor. Further, the responses of elicitors were assessed at three different growth stages under created disease pressure by artificial inoculation.

From the experiments, it is clear that both tomato and brinjal plants are susceptible to *Alternaria solani*. Moreover, the plants can be affected at any growth stage.

Previously, we have demonstrated that both chitosan and yeast elicitor suppress the growth of *Alternaria solani* in culture medium. Moreover, we further demonstrated that chitosan and yeast elicitor can increase the level of endogenous cellular ROS in Arabidopsis (Ref). Therefore, we were interested to investigate the function of chitosan and yeast elicitor in tomato and brinjal plants if they are applied directly on the foliage of growing plants

and simultaneously challenged by *Alternaria solani*.

It is clear that a dose-dependent suppression of *Alternaria* blight was observed by chotosan and yeast elicitor at three growth stages in both tomato and brinjal plants. It can be assumed that application of chitosan and yeast elicitor increased the endogenous ROS which might be the main reason for suppressing disease incidence.

Tomato and brinjal seeds were primed by elicitors to directly antagonize fungal pathogen as well as induce resistance against fungal disease. In case of seed priming with Chitosan Solution, the result showed that Chitosan @ 0.2% significantly reduced seed-borne fungal pathogens. The findings of the present investigation are in agreement with Zheng, et al. [24], who reported that, chitosan coating increased seed germination, plant growth and soybean yield efficiently. We previously reported that some organic elicitors like chitosan and yeast elicitor showed complete inhibition of *mycelial* growth and reduced the incidences of seed-borne fungal pathogens [25-27], 0.1% chitosan help for the growth stimulation of cotton and maize seeds. In case of seed priming with Yeast Elicitor Solution, the result showed that Yeast Elicitor @ 0.2% significantly reduced seed-borne fungal pathogens [28]. Foliar application of yeast extract hold promises for increasing the seed yield and isoflavone content of soybean seeds. In the light of the above recorded results, the present study may suggest that Chitosan and Yeast Elicitor are potential compounds for application in seed disease management. The findings of the present investigation is in conformity with Mondol, et al. [29], where they also reported that Chitosan, Salicylic Acid and Benzoic Acid suppress the growth of *Magnaporthe oryzae Triticum* under *in vitro* condition. For *in vivo* experiment, in tomato plant *Alternaria solani* was selected for artificial inoculation. Abbo, et al. [30], who characterized the causal agent of early blight disease on Solanaceous crops in Sudan as *A. alternata*. After inoculation of tomato plants with the small spots were observed on all tested plants. Significant differences were found in different treatments applied in tomato plants. Anusuya, et al. [31], reported that, Chitosan treated turmeric plants showed increased resistance towards rhizome rot disease caused by *Pythium aphanidermatum. Photchanachai*, et al. [32], reported that, chitosan is effectively reduces the contamination of *Colletotrichum spp*. and improve the quality of chilli seedlings. Mondal, et al. [33], investigated that, foliar application of chitosan at early growth stage to achieve maximum fruit yield in okra. Sathiyabama, et al. [34], reported that, chitosan treatment of groundnut leaves before inoculation reduced the number of leaf lesions, lesion diameter and sporulation of *Puccinia arachidis. Ghaouth*, et al. [35], reported that, chitosan controlled root rot caused by *Pythium aphanidermatum* [36].

Conclusion

Variable reactions were recorded for chitosan and yeast elicitor considering both development stages and kind of harvests. Chitosan performed predominant in tomato plants while yeast elicitor in brinjal plants thinking about both illness occurrence and seriousness. In the two cases, 42 DAT was more appropriate for chitosan and yeast elicitor splash to get most extreme infection concealment. Chitosan and yeast elicitor at 0.2% demonstrated unrivaled execution in diminishing scourge rate and seriousness by Alternaria.

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