Cumin cultivation has received a severe threat from *Fusarium oxysporum f. sp. cumini* and *Alternaria burnsii*, causative agents of wilt and blight diseases respectively, which ravage the total standing crop in field at different stages of life cycle. The disease start and spread are highly dependent on the climate conditions. Application of general or specific fungicides and chemical agents also cannot control their spread in the field. The promising way to control the diseases is by provocation of crop’s own immunity i.e. SAR. Various experiments were conducted to establish the SAR in cumin plants and finally controlling the diseases to avoid crop losses.

**Study of fungal pathogenicity:**

The term pathogenicity is defined as the capacity of a fungus to cause disease. Here in the present study, sterile healthy leaves of cumin plants were incubated with spores of pathogenic fungi, *F. oxysporum* and *A. burnsii* in a sterile petriplates to check their pathogenicity. After 24 hrs of incubation, *F. oxysporum* infected leaves turned yellowish brown and *A. burnsii* infected leaves turned brown whereas leaves incubated with sterile distilled water remained green without showing any symptoms of pathogenicity (Fig. 23A-C). Thus, the selected cultures of both the fungi are potent pathogens for the cumin plants since identical symptoms were observed under the field conditions.

**Morphological study of fungal strains:**

Seven days old cultures of *F. oxysporum f. sp. cumini*, *A. burnsii* and *Trichoderma harzianum* growing on potato dextrose agar were used for the morphological study (Figs. 20C, 20F, 21D). *F. oxysporum f. sp. cumini* takes 4-5 days to grow and cover the whole PDA plate. Initially it grows as white mycelium with no pigmentation in PDA plate. On maturation the fungus liberates toxin and the plate becomes red in colour. The fungus forms sickle shaped macroconidia, ovoid microconidia and chlamydospores. Microconidia is single celled, hyaline and ovoid to ellipsoid, straight or curved and constitutes about 90% of the spores produced. Macroconidia are few in number, mostly 2-3 septate, straight or slightly curved at the apex forming a beak (Fig. 20E).
**Alternaria burnsii** grow as mycelium and covers the whole PDA plate in 3-4 days of inoculation. The mycelial mat shows dark greenish colour upon growth. *A. burnsii* produces conidiophores vertically. Conidia are born at the tip of conidiophores. Conidia are obolavate in shape, having transverse longitudinal or oblique septa. Conidia have long beak and pale dirty brown colour (Fig. 20H).

**Trichoderma harzianum** is comparatively fast growing and covers the plate within 2-3 days of inoculation. The mycelium is green in colour. The organism grows and ramifies as typical fungal hyphae, 5 to 10 µm in diameter. Asexual sporulation occurs as single-celled, usually green, conidia that are released in large numbers. Intercalary resting chlamydospores are also formed, these are single celled, although two or more chlamydospores may be fused together (Fig. 21F).

The colony of *Bacillus subtilis* is cream in colour, flat and circular with undulated margin on nutrient agar. They are gram positive organisms. The cells were stained using Gram’s staining procedure. When observed under microscope, the cells of *B. subtilis* were rod shaped and violet in colour (Fig. 21C) which confirms the gram positive nature of the selected bacterium.

**Estimation of protein in fungal culture filtrates (FCFs) and cell free culture filtrate (CCF):**

Estimation of protein concentration by using Folin- Lowry’s method of selected fungal strains growing in potato dextrose broth (PDB) (Figs. 20D, 20G, 21E) was performed for 28 days. The highest protein concentration was measured on 15th and 23rd day of incubated culture of *F. oxysporum f. sp. cumini* and *A. burnsii* in PDB medium (Figs. 22A, 22B) respectively. In FCF of *F. oxysporum f. sp. cumini* and *A. burnsii*, highest protein concentration 3.0 mg/ml and 1.12 mg/ml was measured respectively. In *T. harzianum* FCF the highest protein concentration was 1.10 mg/ml on 24th day of incubation in PDB medium (Fig. 22D). The culture filtrates with highest protein concentration were selected for further experimental work.

The *Bacillus subtilis* was grown in nutrient broth for one week (NB) (Fig. 21B), in which highest protein concentration 3.90 mg/ml was recorded in 24 hrs old
culture (Fig. 22C). The CCF collected after 24 hrs of growth of the bacterium in nutrient broth and was selected for the experiments.

**Purification and characterization of elicitors:**

On the basis of higher protein concentration, 15\textsuperscript{th} and 23\textsuperscript{rd} day harvested crude fungal culture filtrate of *F. oxysporum f. sp. cumini* and *A. burnsii* respectively was used for ammonium sulphate (AMS) precipitation for the purification of elicitor active compound. The total protein of the crude fungal culture filtrate (FCF) of both fungi was precipitated by using different concentrations of ammonium sulphate (10%, 20% up to 100%) separately and observed the total protein concentration of different fractions and also estimated the PAL activity and total protein concentration after 1 day of foliar application to pot grown cumin plants. The higher total protein concentration and PAL enzyme activity was observed in 80% and 90% ammonium sulphate precipitated FCFs of *F. oxysporum f. sp. cumini* and *A. burnsii* (Table 8) respectively.

The ammonium sulphate precipitated FCFs of both pathogenic fungi were kept in dialysis bags separately for desalting in phosphate buffer (pH 7.0) overnight, in freezing condition on a magnetic stirrer for further purification. Dialyzed samples were further purified using column chromatography using Sephadex G-50 gel filtration column (GFC). Column purified seventh fraction of *F. oxysporum f. sp. cumini* and *A. burnsii* FCF was found to contain active compound as they showed highest absorption peak at 280 nm. Induction of PAL and total protein concentration was measured upon the application of active purified fractions separately after 1 DAT on 20 day old pot grown cumin plants.

The 7\textsuperscript{th} purified fraction of *F. oxysporum f. Sp. cumini* FCF showed 4.9 fold higher PAL activity and 3.1 fold increase in protein concentration and *A. burnsii* FCF showed 3.7 fold increase in PAL activity and 2.3 fold increase in protein concentration than control plants treated with distilled water.
Table 8: Total protein concentration of FCFs after precipitation with different concentration of ammonium sulphate (AMS) and induction of PAL activity and protein concentration in cumin leaf treated with crude, AMS precipitated and dialyzed fungal culture filtrate.

<table>
<thead>
<tr>
<th>Fungal Culture Filtrate precipitated with AMS (% conc)</th>
<th>Fusarium oxysporum f. sp. cumini</th>
<th>Alternaria burnsii</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein con. of FCF (mg/ml)</td>
<td>PAL activity in treated plants (U/ml/gft)</td>
</tr>
<tr>
<td>Crude FCF</td>
<td>3.1±0.61</td>
<td>1.726±0.11</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>0.09±0.03</td>
</tr>
<tr>
<td></td>
<td>20%</td>
<td>0.11±0.003</td>
</tr>
<tr>
<td></td>
<td>30%</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td></td>
<td>40%</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>0.43±0.05</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>0.72±0.10</td>
</tr>
<tr>
<td>70%</td>
<td>1.14±0.04</td>
<td>0.74±0.05</td>
</tr>
<tr>
<td></td>
<td>80%</td>
<td>0.61±0.17</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>0.40±0.06</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>0.20±0.12</td>
</tr>
<tr>
<td>70% AMS after dialysis</td>
<td>0.87±0.04</td>
<td>0.70±0.003</td>
</tr>
</tbody>
</table>

Crude, dialyzed and column purified 7th fraction of both FOFCF and ABFCF elicitor sample were loaded and separated using SDS-PAGE individually. The gels were later stained with PAS to determine the nature of elicitor active compound. Crude culture filtrates and ammonium sulphate precipitated samples have shown the
presence of various proteins with molecular weight ranges between 14.3-97.4 kDa. But column purified elicitor sample revealed the presence of 29.0 kDa molecular weight active fraction of protein in both samples. The molecular weight of the active protein band lies in the range of the PR protein family-3 and hence it can be suggested that the nature of the principal protein compound would be endochitinases. This protein when stained with Periodic acid and Schiff’s reagent (PAS) showed the appearance of pinkish orange color protein band which confirms that the elicitor protein present in both the fungal FCFs is glycoprotein (Fig. 23D & E).

Many researchers have purified the active compound/s present in either bacterial or fungal origin elicitors and also evaluated their effect on various crop plants. Stekoll and West (1978) revealed that glycoprotein purified from the fungus Rhizopus stolonifer boost the resistance by inducing phytoalexins in castor bean. Schaffrath et al. (1995) purified glycoprotein from the mycelial walls of Pyricularia oryzae, causal agent of rice blast and observed induced resistance in rice plants against pathogens. Bailliul et al. (1995) observed hypersensitive response in tobacco plant when treated with a glycoprotein purified from Phytophthora megasperma. Mishra et al. (2009) purified glycoprotein from Phytophthora colocasiae and its infiltration to Colocasia esculenta leaves induced HR activity. A glycoprotein synthesized from yeast induced phytoalexin accumulation in sorghum mesocotyls (Wulff and Pascholati, 1999) and PAL synthesis in tomato cell suspension cultures (Glazebrook, 2005). Ricci (1997) isolated Elicitin from several species of Phytophthora and observed increase in PR- protein activity in the leaves when applied to tobacco plants and resistance against the Phytophthora var nicotianae. Picard et al. (2000) isolated a 10kDa Elicitin like protein termed Oligandrin from culture filtrate of Phytophthora oligandrum which induced plant defense reactions upon challenged with Phytophthora parasitica in tomato plants. In red pepper plants Sriram et al. (2009) reported the glucan elicitor extracted from Trichoderma harzianum isolate found to be superior in biocontrol potential which induced glucanase activity and phenol content. Bariya et al. (2011) purified a 10 kDa protein (infestin) from the fungal culture filtrate of Phytophthora infestans and its treatment to Solanum tuberosum plants induce peroxidase, glutathione reductase and catalase enzyme activities and HR response. Yang et al. (2009) also identified the presence of glycoprotein elicitor in mycelium culture of Alternaria tenuissima which can induce plant resistance and become a
potential agent for biological control of plant diseases. This study also revealed the presence of a glycoprotein (29 kDa protein) in the FCFs of both *F. oxysporum f. sp. cumini* and *A. burnsii* which may be responsible for induction of different defense enzymes upon application to the cumin plants.

**Standardization of time and concentration of FCFs and CCF for treatment:**

The seeds were germinated in various concentrations (0.5%, 1%, 5%, 10% up to 50%) of selected fungal culture filtrates and checked phenylalanine ammonia lyase and peroxidase enzyme activities which varied among the various concentrations (Figs. 5A-D). The highest PAL and POX activities were observed in 10% concentration of *F. oxysporum f. sp. cumini* and 5% concentration of *A. burnsii* culture filtrate treated seeds (Figs. 24A-B; 25A-B; 26A-B). The highest PAL and POX activity was observed in 5% and 20% concentration of *B. subtilis* and *T. harzianum* culture filtrate treated seeds respectively (Fig. 24A-B). The concentration of culture filtrates in which highest activity was noted subsequently selected for further experimentation.

The treated seeds and germinated seedlings were harvested at different time intervals (0 hr, 30 min, 1 hr, 3 hrs, 6 hrs, 12 hrs, 24 hrs, 48 hrs upto 144 hrs) and estimated the PAL, POX enzyme activities, which varied among different time intervals and observed significantly higher activity than in control. Highest induction of the PAL and POX enzyme activities was found in 10% FCF of *F. oxysporum f. sp. cumini* at 3 hrs, in 5% *A. burnsii* FCF at 1 hr and in MIX FCF at 30 min after the seed treatment (Figs. 24C, 25C-D) at different time intervals. Likewise, 20% FCF of *T. harzianum* at 24 hrs and 5% CCF of *B. subtilis* at 1 hour of treatment showed highest induced PAL and POX activity (Fig. 24C-D) in treated seeds. Therefore, the same concentration and time mentioned above was used for all treatments during the study.

Concentrations at 0.1% and 1% headline, 0.5% and 1% monitor were chosen randomly for experimentation. Among these 0.1% headline and 1.0% monitor were found to be more active and therefore used in the study at the later stages. Treatment with selected concentrations of headline resulted in induction of PAL and POX activity at 30 min and monitor treated plants at 6 hrs (Fig. 26C-D) which was selected for further experimentation.
Results and Discussion

Seed treatment with 5% *B. subtilis* CCF showed highest induction of various enzymes activities compared to other treatments in case of pot and experimental plot assay. Although seed treatment with 10% FOFCF and 5% ABFCF also almost showed similar induction of enzyme activities in pot and experimental plot grown plants but their best response were observed in foliar spray treatment when compared with *B. subtilis* CCF treatment. Moreover, giving treatment to seeds in a large quantity is bit complex as adhesion of the agent was not happening evenly as of marketed product where they use various slurry agents. Among 0.1% and 1.0% headline, 0.5% and 1.0% monitor, 0.1% headline showed considerable response for induction of various enzyme activities. Therefore, in this study, foliar spray treatments with 10% FOFCF, 5% ABFCF, 0.1% Headline were selected and used as per the design of experimentations at farmer’s field.

**Seed germination Assay**

Seed germination assay was performed in lab conditions in petriplates and pots containing soil to check the germination rate and vigour following seed treatment with different elicitors. Both GC-2 and GC-4 treated seeds showed highest germination rate with 10% FOFCF treatment followed by 5% ABFCF and MIX FCF treatment (Table 9) while control seeds showed less germination rate. The results strongly indicate that elicitors are involved in enhancing the germination vigour of the seeds.

Among different fungal elicitor treated seeds, treatment with 10% FOFCF showed earlier germination by emergence of radical on 4 and 3 DAT in GC-2 and GC-4 variety respectively (Table 9) as compared to control seeds. After germination, length of the seedlings was also found to be gradually increased in treated seeds than untreated control seeds (Fig. 27A-F).
Table 9: Germination rate and vigour in GC-2 and GC-4 variety of seeds with various elicitor treatments.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Germination Rate</th>
<th>Emergence of radical (DAT)</th>
<th>Emergence of plumule (DAT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td>Control</td>
<td>66.67%</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>86.67%</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>80%</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>80%</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>73.34%</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>GC-4</td>
<td>10% FOFCF</td>
<td>99.93%</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>86.67%</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>99.90%</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

Elicitors are known to induce germination in treated seeds by inducing salicylic acid production which in turn leads to induction of auxin synthesis, a plant growth hormone which promotes enhanced radical formation (Linden and Stoner, 2005). The seeds treated with 10% FOFCF, 5% ABFCF and MIX FCF and kept in sterile petriplate with moist Whatman filter paper showed marked difference in the length of radical emergence 7 days after treatment. Seeds of both the varieties treated with MIX FCF showed the increased length of radical than other treatments (Table 10). This shows the combined effect of both FCFs on germination of cumin seeds. Control seeds of both varieties showed reduced germination rate, vigour and growth as compared to other treatments.
Table 10: Effect of FOFCF, ABFCF and MIX FCF on seed germination and radical length after emergence

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Length of Radical</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 DAT</td>
</tr>
<tr>
<td>GC-2</td>
<td>Control</td>
<td>1.42±0.19</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>2.36±0.27</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>2.32±0.39</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>2.10±0.43</td>
</tr>
<tr>
<td>GC-4</td>
<td>Control</td>
<td>1.60±0.22</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>1.96±0.36</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>1.86±0.40</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>2.54±0.58</td>
</tr>
</tbody>
</table>

Fungal elicitors have role in inducing plant’s growth and development which leads to increased yield at the end. In the present study, germination rate was checked after 21 days of seed treatment with standardized concentration of *F. oxysporum*, *A. burnsii* and MIX FCFs and allowed to germinate in pots containing garden soil. In GC-2 variety, maximum germination rate was observed in seeds treated with 10% FOFCF (93.33%) and in GC-4 variety, 10% FOFCF and MIX FCF treated seeds showed highest germination rate (93.33%) compared to other treatments (Table 11). Once again combination of both FCF showed adequate response in seed germination that confirms the synergistic effect on fungal elicitors on growth. Control seeds treated with distilled water showed lowest germination rate in both the varieties (Fig. 28A-B).
Table 11: Germination rate of cumin plants grown in normal soil treated with elicitors after 21 days of sowing

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Number of seeds sown/2 pots</th>
<th>Survived no. of seedlings</th>
<th>Germination Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>18</td>
<td>60%</td>
</tr>
<tr>
<td>GC-2</td>
<td>10% FOFCF</td>
<td>30</td>
<td>28</td>
<td>93.33%</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>30</td>
<td>22</td>
<td>73.33%</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>30</td>
<td>27</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>20</td>
<td>66.66%</td>
</tr>
<tr>
<td>GC-4</td>
<td>10% FOFCF</td>
<td>30</td>
<td>28</td>
<td>93.33%</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>30</td>
<td>26</td>
<td>86.66%</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>30</td>
<td>28</td>
<td>93.33%</td>
</tr>
</tbody>
</table>

Several researchers have shown the enormous effect of bioelicitors on seed germination and growth. Compared to controls, elicitor treated seeds germinated early and were more vigorous, which ultimately results in statistically increased crop performance and higher yields (Linden and Stoner, 2005). The culture filtrates of *T. longibrachiatum*, *T. harzianum* and *T. viride* had a favourable influence on the germination of spinach seed while the filtrates of *T. longibrachiatum* and *T. harzianum* also stimulated the germination of red beet, tomato and chicory seeds and in most cases it was observed that the filtrates increased the speed of germination (Celar and Valic, 2005). Application of *Trichoderma spp.* also showed faster germination and increases in percentage of emergency, plant height, leaf area and dry weight in tomato pea and radish (Lindsey and Baker 1967; Baker et al., 1984; Chang et al., 1986; Kleifeld and Chet, 1992; Inbar et al., 1996).

Many scientists have reported the increased rate of germination and plant growth using several chemicals, biocontrol agents or bioelicitors. James and Richard (2008) reported that the proprietary elicitor, YEA derived from chitin/chitosan showed improved seed germination rate in many vegetables compared to control. It
impacts to the receptor on the cell surface and initiate molecular level signal transduction processes such as induction of β-1,3 glucanase in treated seeds which is associated with improvement in germination rate. They also observed that in tomato hypocotyls 87% greater length than control and 14% more germination than control in treated seeds of sweet corn. Du et al., (2000) used natri-tetraborate to enhance the blast resistance and found increased root and coleoptile length and grain yield up to 10-12% more compared to control. Karthikeyan et al., (2007) found that the use of diazotrophs *Azospirillium* and *Azotobacter* increased the germination rate up to 70% against 35% recorded in untreated control along with increase in superoxide dimutase, POX and catalase activities. Mao et al. (1997) noted the increase in plant height, fresh weight and decrease in root rot severity in treated seeds with pre-infiltration and treatment with biocontrol agents such as *Tichoderma viride* and *Glicladium virens* in order to reduce damping-off caused by species of *Pythium* and *Fusarium* in corn. In this study also treatment of 10% FOFCF and 5% ABFCF resulted in best cumin seed germination and increased growth in comparison with other treatments and control.

In the present study, similar results i.e. higher rate of germination and good growth of plants treated with FCFs was observed. This shows that the elicitor triggers biochemical pathway that helps cumin plants for their better growth and development. Looking into the results achieved, seed as well as foliar spray treatment experiments were conducted to confirm the better mode of treatment as well as agent for treatment that controls the disease spread and offers long lasting immunity to cumin plants.

**Triggering SAR in cumin plants:**

➤ **Pot Assay- Soil infestation study:**

**Experiment 1:**

In pot assay, the seeds of both GC-2 and GC-4 varieties showed good germination and growth upon treatment with 10% *F. oxysporum f. sp. cumini* FCF and 5% *B. subtilis* CCF and FCF of *T. harzianum* compared to control plants. The treated plants were greener and healthy even in *F. oxysporum f. sp. cumini* infested soil (Fig. 29A-F).
Experiment 2:

Seeds of both the varieties of cumin i.e. GC-2 and GC-4 were treated with standardized concentration of selected elicitors at specific time period and sown in *F. oxysporum* infested soil and observed the mortality rate after 20 days. The lowest mortality rate was observed in 10% FOFCF treated seeds growing in infested soil among different treatments and the mortality rate observed as 20% and 16% in GC-2 and GC-4 variety plants respectively whereas highest mortality rate was observed in control plants treated with distilled water (Table 12). Among the commercial fungicides used, 0.1% headline showed better response. But 10% FOFCF treated plants were healthy and showed more vigour compared to other treatments and the reason behind it may be the activation of SAR in cumin plants that fights against the pathogen present in the soil.

Table 12: Mortality rate of plants growing in soil infested with *F. oxysporum f. sp. cumini* after 20 days of treatment with various elicitors

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>GC-2</td>
<td>76%</td>
</tr>
<tr>
<td>GC-4</td>
<td>71%</td>
</tr>
</tbody>
</table>

Experiment 3:

Assessment of mortality rate:

In this experiment, seeds of both GC-2 and GC-4 varieties were treated with standardized concentration of elicitors (FOFCF, ABFCF and Mix FCF) for selected time period and sown in *F. oxysporum* and *A. burnsii* infested soil separately and observed the mortality rate after 20 days. The lowest mortality rate was observed with 10% FOFCF treated seeds germinated in *F. oxysporum* infested soil than other treatments and control (Fig. 30A-D) (Table 13).
Table 13: Mortality rate of plants growing in soil infested with *F. oxysporum f. sp. cumini* after 20 days of seed treatment with various elicitors

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>GC-2</td>
<td>76%</td>
</tr>
<tr>
<td>GC-4</td>
<td>71%</td>
</tr>
</tbody>
</table>

In contrast, among all the treatments, GC-2 and GC-4 seeds treated with 5% ABFCF resulted in lowest mortality rate of 20% and 18% respectively (Table 14), in *A. burnsii* infested soil whereas control seeds showed 82% and 63% mortality which being the highest among all the treatments (Fig. 28A-D).

Table 14: Mortality rate of plants growing in soil infested with *A. burnsii* after 20 days of seed treatment with various elicitors

<table>
<thead>
<tr>
<th>Variety</th>
<th>Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>GC-2</td>
<td>82%</td>
</tr>
<tr>
<td>GC-4</td>
<td>63%</td>
</tr>
</tbody>
</table>

It is presumed that the treatment with elicitors induces the resistance mechanism in the seedlings and plants which eventually boost the production of defense related enzymes, phytoalexins, other secondary metabolites which directly or indirectly acts upon pathogen and restrict their growth and infection. Because of this reason, more vigour and less mortality is seen in elicitor treated plants in comparison with control plants growing in fungal infested soil.
Results and Discussion

- **Experimental plot assay:**

  **Seed treatment assay:**

  The GC-2 and GC-4 variety seeds were treated with FOFCF, *T. harzianum* FCF and *B. subtilis* CCF and grown in experimental plots. After 25 day of growth, quantification of enzymes (presented below) such as phenylalanine ammonia lyase (PAL), peroxidase (POX), polyphenol oxidase (PPO), β-1, 3 glucanase, catalase, total protein concentration, total phenol concentration along with protein and phenol profiling was done. It was seen that FOFCF and *B. subtilis* CCF treated seeds grown plants showed higher induction of defense related enzymes among all treatments. Among these two treatments, *B. subtilis* CCF treatment showed highest induction of all enzymes and biochemical parameters.

  **Foliar spray application assay:**

  **Experiment 1:**

  Foliar spray treatment was given with 10% FOFCF, 20% *T. harzianum* FCF and 5% *B. subtilis* CCF to both the variety of 25 days old cumin plants. FOFCF treated plants showed highest induction of PAL, POX, PPO, β-1, 3 glucanase, catalase, total protein and total phenol concentration among different treatments. Protein and phenol profiling was also performed from the plant samples collected after 25 days of growth. More number of induced as well as prominent bands of protein and phenols were observed in the study from the sample collected after the treatment with 10% FOFCF compared to other treatments. It is assumed that, these induced bands have role in boosting the immunity of the cumin plants.

  **Experiment 2:**

  Single spray treatment was given to twenty-five-days old plants of GC-2 and GC-4 varieties plants with 10% FOFCF, 0.1% and 1.0% headline; and 0.5% and 1.0% monitor. The leaves were collected for the preparation of enzyme extract daily for one week to analyze the defense related enzymes activities such as PAL, POX, β-1, 3 glucanase, PPO, catalase, total protein concentration, total phenol concentration along with protein and phenol profiling, nitrate reductase (NR), nitrite reductase (NiR),
reducing and non-reducing sugar content. Among all selected treatments, 10% FOFCF treated plant samples have shown highest induced activity of various enzymes compared to other treatment. 0.1% headline treatment have adequately induced the enzyme activities but not as of induced by FOFCF. Control plants have shown lowest response in inducing defense related enzyme activities.

**Experiment 3:**

Twenty five days old GC-2 and GC-4 variety of cumin plants grown in experimental plots were given foliar spray treatment with 10% *F. oxysporum* FCF, 5% *A. burnsii* and MIX FCF which showed induction of defense related enzymes and appeared to be healthy compared to control plants. After an interval of another 25 days, second foliar spray treatment was given with the respective elicitors to boost the plant defense mechanisms. Higher concentration of various defense related enzymes was estimated in both the varieties in each treatment. FOFCF treatment showed maximum induction of defense related enzymes during the 1st foliar spray, while in 2nd spray, induction of defense related enzymes were seen in ABFCF treated plants. On the other hand, MIX FCF treatment showed moderate induction in both the varieties as compared to FOFCF and ABFCF treatments.

Moreover, plants treated with 10% FOFCF showed early flowering as compared to other treatments. In control plants flowering was delayed by 10 to 14 days, which indicates that elicitors also promote rapid growth and development of the plants. Moreover, plants treated with FOFCF were much greener and healthy compared to control plants. Headline treated plants were also healthy, but 3-5 days delayed flowering and less vigour was observed in comparison with FOFCF treated plants.

**Quantification of defense related enzymes from the above experiments:**

Utilization of plant’s own defense mechanism is the area of current interest in management of pathogenic diseases. In SAR, certain resistance genes translate into the proteins that confer long lasting induced immunity to plants. The defense gene products include peroxidase (POX), polyphenol oxidase (PPO), catalase and phenylalanine ammonia lyase (PAL) involved in phytoalexins and phenolics.
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Various defense related enzymes and other biochemical parameters were estimated in samples collected for 7 days from plants growing in infested soil, experimental plot and farmer’s field after treatment with various elicitors.

Leaf samples were collected from the plants growing in the experimental plots (seed treatment, foliar spray treatment) (Fig. 31A-C) and farmer’s fields (Fig. 31D-G) at different time intervals and estimated various induced defense related enzymes in the treated plants and compared with control plants. The induction of various enzyme activities varied among various elicitor treatments at different time interval throughout the experiment. The elicitor treated plants showed more response in comparison with control plants.

Phenylalanine ammonia lyase (PAL) activity:

Phenylalanine ammonia lyase (PAL) is the first enzyme in the phenyl propenoid biosynthetic pathway. PAL activity is an extremely sensitive indicator of fungal challenge and stress condition. PAL activity is associated with biosynthesis of toxic metabolites such as phytoalexins, phenols, lignins and salicylic acid in plant defense pathways (Mauch-Mani and Slusarenko, 1996).

In soil infestation experiment 1 of pot assay of the present study, the plants of both GC-2 (Fig. 32A) and GC-4 (Fig. 34A) varieties growing in F. oxysporum f. sp. cumini infested soil showed highest induction of PAL at 120 hrs when treated with 10% F. oxysporum f. sp. cumini FCF and 5% B. subtilis CCF whereas the plants treated with 20% FCF of T. harzianum showed highest induction of PAL at 48 hrs. But the highest increase in fold count of PAL enzyme activity was found in plants treated with B. subtilis i.e., 2.03 fold in GC-2 plants and 2.19 fold in GC-4 plants (Table 18) in comparison with other treatments.

In the experiment 3 of pot assay, during soil infestation study, seeds grown in F. oxysporum f. sp. cumini infested soil after the treatment with 10% FOFCF, 5% ABFCF and MIX FCF showed highest PAL activity with 10% FOFCF treatment at 120 hrs (Figs. 37A, 40A) which is 1.93 fold in both the varieties (Table 19) while 5% ABFCF exhibited 1.81 and 1.69 fold induction at 96 hrs and treatment with MIX FCF resulted in 1.74 and 1.76 fold induction of PAL activity at 120 hrs in GC-2 and GC-4.
varieties respectively in comparison with control. These results indicate that FOFCF treatment is most effective in induction of PAL compared to other treatments. Similarly, in plants growing in *A. burnsii* infested soil highest PAL induction observed in both the varieties seeds treated with 10% FOFCF resulting 2.64 and 2.51 fold increase in PAL activity as compared to 5% ABFCF treatment (Figs. 43A, 46A) which showed 2.55 and 2.10 increase in fold activity while MIX FCF treated plants showed 2.54 and 2.30 fold increase in activity in GC-2 and GC-4 varieties respectively (Table 20).

Seed treatment assay performed in experimental plots, the plants of both the varieties were grown after seed treatment with FOFCF, *T. harzianum* FCF and *B. subtilis* CCF. They showed highest activity of PAL at 120 hrs in seeds treated with FOFCF and *B. subtilis* CCF. The activity of PAL was highest at 48 hrs with *T. harzianum* seed treatment in both the varieties (Figs. 51A, 53A). The highest increase in fold count 1.69 and 1.61 in GC-2 and GC-4 varieties respectively was observed with *B. subtilis* treatment as compared to other two treatments (Table 21).

In experiment 1 of foliar spray treatment with 10% FOFCF, 20% *T. harzianum* FCF and 5% *B. subtilis* CCF to the plants growing in experimental plots, the highest activity of PAL was observed at 120 hrs with *F. oxysporum f. sp. cumini* and *B. subtilis* whereas with *T. harzianum* treatment at 96 hrs (Figs. 56A, 58A). Highest fold count of PAL activity, 2.05 fold in GC-2 and 2.23 fold in GC-4, was observed in plants treated with *F. oxysporum f. sp. cumini* FCF (Table 22). The PAL activity was observed highest in all the treatments throughout the experiment when compared with control plants.

In second foliar spray application experiment performed in experimental plot, PAL activity was gradually increased in treated plants compared to untreated control plants in both varieties of cumin. In this study, PAL activity was highest at 3.25 and 3.28 fold in GC-2 and GC-4 varieties respectively (Figs. 61A, 64A) (Table 23) after 10% FOFCF treatment at 120 hrs. While 0.1% headline treated plants exhibited 2.71 and 2.73 fold induction of PAL at 72 hrs and plants treated with 1% monitor resulted in 2.51 and 2.42 fold induction of PAL activity at 48 hrs in GC-2 and GC-4 varieties respectively compared to untreated control. These results indicate that FOFCF treatment is most effective in induction of PAL compared to treatments with headline
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and monitor. The PAL activity remained high in treated plants compared to untreated plants in both the varieties throughout the experimentation.

In third experimentation, conducted in plot, 1st foliar spray treatment was given to the 21 days old cumin plants with 10% FOFCF, 5% ABFCF and MIX FCF and same treatment was given on 51st day as second application. FOFCF treatment in both the spray treatments showed highest induction of PAL activity than other treatments. Induction in PAL activity after FOFCF and MIX FCF treatment was seen after 6 days of the treatment and it was higher on 5th day of the treatment in case of ABFCF treatment during 1st foliar spray application. 1.65 and 2.22 fold increase in PAL activity have been observed in GC-2 and GC-4 varieties during 1st foliar spray after 10% FOFCF treatment while after 2nd spray, 1.75 and 1.48 increase in fold count of PAL activity was obtained in 5% ABFCF treated plants on 5th day of the treatment being the highest (Table 24 & 25) compared to other treatments (Figs. 68A, 71A). Plants treated with fungal elicitors showed good vigour. Flowering was also initiated early in fungal elicitor treated plants compared to control.

PAL is involved in synthesis of lignin precursors and isoflavonoids derived phytoalexins and chalose synthase (Lawton and Lamb, 1996). De-meyer et al., (1999) reported that the rhizosphere colonization by Pseudomonas aeruginosa 7NSK2 activated PAL in bean root and increased salicylic acid level. Studies performed by Chen et al., (2000) and Karthikeyan et al. (2005) have shown that PAL activity is induced in onion plant upon treatment with P. fluorescens. Davis and Ausubel (1989) reported the induction of PAL activity which reached maximum level at 8 to 10 hrs after elicitor- bacterial PGA lyase treatment in Arabidopsis. Induction of systemic disease resistance and pathogen defense responses were also reported by He et al. (2002) in terms of PAL activity in Asparagus officinalis inoculated with non-pathogenic strains of F. oxysporum. Basha and Chatterjee (2007) observed the increase in PAL activity in foliar spray treatment of growth promoting rhizobacterial strains Pseudomonas florescences and P. aeruginosa in wheat plant. Induction in PAL activity after 4 hrs was also observed by Takenaka et al., (2003) in sugar beet and wheat seedlings treated with both types of cell wall protein fractions from the mycoparasite Pythium oligandrum. Raju et al., (2008) observed higher levels of phenylalanine ammonia-lyase (PAL) in roots and shoots of resistant cultivar of
chickpea than that of susceptible cultivar on treatment with elicitors and pathogen. Kartikeyen et al., (2006) studied the effect of soil application of biocontrol agents like *Pseudomonas florescence*, *Trichoderma viride* and *T. harzianum* in combination leads to the induction of SAR by triggering phenyl propanoid pathway to induce defense related enzyme like PAL which reached at maximum level on 3rd day in treated coconut plants against *Ganoderma lucidum*. Estrada et al., (2009) studied the fungal disease in netted melon fruit with the induction of natural defense response (NDR) after the fruit treatment with bioelicitor and inoculated with *Fusarium oxysporum*. After elicitor treatment, higher activity of PAL was seen in melon on 4th day of storage compared to control. Novel oligosaccharides isolated from *Fusarium oxysporum* L. have also been shown to rapidly induce PAL activity in Rubus cells (Lazar et al., 2004). Nandini et al. (2010) reported the substantial increase in PAL activity at 96 hours after treatment with fungal elicitors in different varieties of groundnut plants. PAL induction was also reported by Mahapatra et al., (2015) in *Zea mays* after 4th day of treatment with *Aspergillus flavus* and *A. parasiticus* FCFs. Induction of PAL in this study also support the induction of defense response in cumin plants treated with selected elicitors. Among all selected elicitors in the present study, FOFCF showed best results when applied as first foliar spray and ABFCF as second spray. *B. subtilis* CCF have also shown better results when applied as seed treatment but looking in to the efficacy of the treatment and provocation of defense, application of pathogenic FCFs suits more in cumin to control diseases. Use of *T. harzianum* FCF is also not feasible as it needs in large amount and doesn’t induce the defense related enzymes at adequate level. Moreover, foliar application of fungal elicitors has provoked defense response at significant level in comparison with seed treatment. Thus, use of pathogenic fungal elicitors via foliar application at appropriate time of growth can apply at field level. Looking in to these facts, field study was conducted.

**Peroxidase (POX) activity:**

Plant peroxidases (POX) are ubiquitous, heme containing glycoproteins that catalyze oxidation of diverse organic and inorganic substances at the expense of hydrogen peroxide (H₂O₂) (Gomez-Vasquez et al., 2004). Peroxidase was recorded as one of the first enzymes responding and providing fast defense against plant
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POX activity was seen to be induced in samples obtained from the plants of both the varieties grown after seed treatment with FOFCF, *T. harzianum* FCF, *B. subtilis* CCF in experimental plots (Fig. 51B, 53B). POX activity was higher in all pathogens (Sulman et al., 2001; Luhova et al., 2003). POX is involved in cross-linking extension molecules to form lignin (Brisson et al., 1994) and play specific role in the hypersensitive containment of the pathogen (Peng and Kuc, 1992). Peroxidases have been implicated in a variety of defense related processes, including the hypersensitive response, lignification, cross-linking of phenolics and glycoproteins, suberization, auxin catabolism, wound healing and phytoalexin production (Wojtaszek, 1997; Hiraga et al., 2001). Increases in POX activity are often associated with a progressive incorporation of phenolic compounds within the cell wall during incompatible plant–microbe/elicitor interactions.

In experiment 1, the samples obtained from plants of both the varieties grown in *F. oxysporum f. sp. cuminii* infested soil in pot assay showed highest activity of POX at 120 hrs after treatment with FOFCF and *B. subtilis* CCF, whereas *T. harzianum* FCF treatment showed activity after 48 hrs (Figs.32B, 34B). The highest increase in fold count activity was found in seeds treated with *B. subtilis* CCF i.e. 2.37 and 2.13 folds in GC-2 and GC-4 respectively (Table 18).

In the experiment 3 of soil infestation study, transient increase was noticed in peroxidase activity in 10% FOFCF and MIX FCF treated plants. It was maximum at 120 hrs after the treatment. This increase was followed by a decrease in activity after 120 hrs but was higher than control throughout the experiment. Similar results were recorded with the treatment of 5% ABFCF which induced maximum activity at 96 hrs (Figs. 37B, 40B) in both the varieties. Peroxidase activity was studied in cumin seeds treated with elicitors and grown in soil infested with *F. oxysporum f. sp. cuminii* and *A. burnsii* separately. Both the seed varieties treated with 10% FOFCF showed maximum increased fold count of 2.10 (Fig. 37B) and 2.04 fold (Fig. 40B) in *F. oxysporum f. sp. cuminii* infested soil which is higher than all the other elicitor treatments (Table 19). Similar results were obtained in *A. burnsii* infested soil, with 2.74 and 2.60 fold increase in POX activity with 10% FOFCF treatment in GC-2 and GC-4 variety (Table 20) but highest activity was exhibited by ABFCF treatment at 96 hrs (Figs. 43B, 46B).
treatment than control plants. The highest fold count of POX activity 2.38 was in GC-2 variety and 1.75 in GC-4 variety after seed treatment with *B. subtilis* CCF after 120 hrs (Table 21). Induction in POX activity was seen at 120 hrs and 48 hrs in FOFCF and *T. harzianum* FCF treated plants respectively.

The highest POX activity was found at 120 hrs with FCF of *F. oxysporum* f. *sp. cumini* and CCF of *B. subtilis* in foliar spray treated plants in experiment 1. The POX activity was highest at 96 hrs with FCF treatment of *T. harzianum* (Fig. 56B, 58B). The highest increase in fold count in POX activity was observed with treatment of FOFCF i.e. 2.17 and 2.88 in GC-2 and GC-4 respectively (Table 22). In all the treatments the POX activity remained high throughout the experiment compared with control plants.

In the second foliar spray experiment performed in experimental plot, the highest fold increase in POX activity 4.21 was recorded with 10% FOFCF treatment in both varieties at 120 hrs (Figs. 61B, 64B) (Table 23) compared to other treatments i.e. as 0.1 and 1.0% headline, 0.5 and 1.0% monitor as well as control. Induction of POX activity in cumin plants was observed after treatment with 0.1 and 1.0% headline at 48 hours where as in monitor treated plants induction was seen after 72 hrs.

In third experiment, two foliar spray treatments were given with FOFCF, ABFCF and MIX FCF. Highest POX activity after FOFCF treatment in 1\textsuperscript{st} foliar spray was recorded compared to other treatments. 1.50 and 1.38 fold increase in POX activity was observed with 10% FOFCF treatment after 1\textsuperscript{st} foliar spray in GC-2 and GC-4 plants respectively on 6\textsuperscript{th} day of the treatment as compared to 1.48 and 1.28 fold increase with 5% ABFCF treatment on 5\textsuperscript{th} day of the treatment while MIX FCF treatment resulted in 1.42 and 1.21 fold increase in activity after 1\textsuperscript{st} spray on 6\textsuperscript{th} day of the treatment (Table 24). In 2\textsuperscript{nd} foliar spray, highest fold increase in POX activity has been exhibited by 5% ABFCF treatment on 5\textsuperscript{th} day of the treatment giving 1.65 and 1.50 fold increase in GC-2 and GC-4 variety plants respectively (Table 25) (Figs. 68B, 71B).

Mwangi et al. (2008) reported the stimulation of peroxidase activity by rhizobacteria isolates *Pseudomonas fluorescens* T58, *P. putida* 53 and *Bacillus*
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`sphaericus` B43 against *Fusarium* wilt in tomato. He et al. (2001) observed that the POX activity increased rapidly by 2.5 to 3.5 fold in roots of *Asparagus officinalis* treated with nonpathogenic isolates of *Fusarium oxysporum* (npFo). Inoculation of bean hypocotyls with a nonpathogenic binucleate *Rhizoctonia* (BNR) also induced peroxidase activity by 2.0 fold (Xue et al. 1998). Baysal et al. (2003) have reported the induction of POX activity using acibenzolar-S-methyl (chemical agent) in tomato seedlings against bacterial canker caused by *Clavibacter michiganensis ssp. michiganensi*. Bashan et al., (1995) have also noticed increased POX activity upon infection of *Pseudomonas syringae pv. tomato* in tomato. Bariya et al. (2011) found the increased activity of POX enzyme in different varieties of *Solanum tuberosum* by pure and crude elicitor treatment. Plant peroxidase has been proposed to play a major role in plants resistance to bacterial, fungal and viral diseases in plants. Udomeprasert and Attathom (1991) reported the positive correlation in increase concentration of peroxidase activity from resistance of plant after the infection or elicitation. Kawano (2003) observed that extracellular secreted plant peroxidase (POX) are considered to catalyze the generation of reactive oxygen species (ROS) coupled to oxidation of plant hormone indole-3-acetic acid (IAA) and defense related compounds such as salicylic acid (SA), aromatic monoamines (AMAs) and chit oligosaccharides (COSs) after the elicitor treatment. Vasquez et al. (2004) reported the role of POX in cassava and observed increase in POX activity to four-fold in cells after 48hrs post-elicitation. Vidhyasekaran et al., (2002) found the role of an elicitor molecule separated from *Macrophomina phaseolina*, the root rot pathogen of mungbean (*Vigna radiata*) cultivars both resistant and susceptible to the pathogen and observed rapid increase in activation of peroxidase in the resistant cultivar compared to that of the susceptible one. The results obtained in the present study correlates with the above mentioned studies done by several researchers which confirm the induction of POX activity upon treatment with fungal elicitor and biocontrol agents that eventually induce SAR in cumin plants. Once again foliar application of pathogenic fungal elicitors has shown the best response in inducing the defense response and hence it can be concluded that FOFCF and ABFCF can be used at field level to restrict the disease spread and boost the yield.
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β-1,3 glucanase activity

β-1,3 glucanase is well known PR protein that is constitutively expressed at low levels in plants and can be dramatically induced when plants are infected by fungal, bacterial or viral pathogens. Induction of β-1,3 glucanase activity in response to various pathogen elicitors has been investigated in plants. β-1,3 glucanase degrade the major cell wall components and inhibit fungal growth (Kishore et al., 2006). Plant β-1,3 glucanase is pathogenesis related protein and classified as member of the PR-2 family (Cheong et al., 2000). Vogel and Barz (1993) studied in detail the role of β-1,3 glucanase in plants at physiological and molecular level due to their widespread role in plant defense response. β-1,3 glucanase inductions in several plants have been seen including pea, bean, tomato, tobacco, maize, soya bean, etc. Infection of tomato plants with *Fusarium oxysporum* significantly increased the activity of β-1,3 glucanase and chitinase, these increments gradually increased with increasing the time of infection (Samia, 2007). This induction in metabolic activity of β-1,3 glucanase is the indication of higher antimicrobial activity. Foliar levels of β-1,3 glucanase was also observed to induce about 2 fold in tomato plants compared to the control using benzo(1,2,3)thiadiazole-7-carbothioic acid (S)-methyl ester (BTH) treatment in experiment carried out by Inbar et al., (1998). Incubation of cucumber and *Arabidopsis* with *Colletotrichum orbiculare* have also shown to induce β-1,3 glucanase activity significantly higher level in plants grown in the compost compared to plants grown in the peat mix (Zhang et al., 1998). Moreover this enzyme falls in the group of PR proteins which is important part of systemic acquired resistance. Therefore, induction of higher β-1,3-glucanase activity in treated plants with elicitors can be directly correlated with induction of defense response in plants.

In both the varieties, the activity of β-1,3 glucanase increased at 120 hrs in seeds treated with FOFCF and *B. subtilis* CCF while at 48 hrs in seeds treated with *T. harzianum* FCF and grown in infested soil in 1st experiment of pot assay (Figs. 33A, 35A). The plants in infested soil showed highest increase in fold count of β-1, 3 glucanase activity with *B. subtilis* CCF treatment in both the varieties i.e. 2.73 in GC-2 and 2.89 in GC-4 (Table 18).

3rd experiment of pot assay, both GC-2 and GC-4 varieties grown in infested soil with pathogen after elicitor treatment showed better induction of β-1,3 glucanase
activity. Plants treated with 10% FOFCF and growing in *F. oxysporum* infested soil exhibited highest activity as 2.58 (Fig. 37C) and 2.50 fold (Fig. 40C) increase at 120hrs, while 5% ABFCF treatment showed 2.21 (Fig. 37C) and 2.13 fold (Fig. 40C) β-1,3 glucanase activity at 96hrs in treated plants which is low as compared to FOFCF treatment. MIX FCF showed moderate increase as 2.53 and 2.48 fold enzyme activity for GC-2 and GC-4 variety plants respectively (Table 19). In plants growing in *A. burnsii* infested soil, 2.68 fold increase has been observed in GC-2 variety (Fig. 43C) and 2.59 fold in GC-4 variety plants (Fig. 46C) treated with 5% ABFCF (Table 20).

The plants grown in experimental plot after the seed treatments with FOFCF, *T. harzianum* FCF and *B. subtilis* CCF showed induction of the enzymatic activity. Highest 2.68 and 2.98 fold activity increase in GC-2 and GC-4 varieties respectively was seen in plants grown after seed treatment with *B. subtilis* CCF after 120 hrs (Table 21) (Fig. 52A, 54A). Induction in β-1,3 glucanase activity was observed at 120 hrs and 48 hrs in FOFCF and *T. harzianum* FCF treated plants respectively which was higher than control but not than *B. subtilis* CCF treatment.

The β-1,3 glucanase activity was highest at 120 hrs in *F. oxysporum f. sp. cumini* and *B. subtilis* treated plants while at 96 hrs in *T. harzianum* treated plants of both the varieties growing in the experimental plots (Figs. 57A, 59A). The plants sprayed with *F. oxysporum f. sp. cumini* FCF showed highest increase in fold count of β-1,3 glucanase activity i.e. 3.23 in GC-2 and 3.44 in GC-4 variety among all treatments (Table 22).

After the foliar spray treatment in second experimental plot experiment, 10% FOFCF showed 2.87 and 4.03 fold induction of β-1,3 glucanase activity in GC-2 and GC-4 variety plants respectively was recorded after 120 hrs which was higher than other treatments including control (Table 23) (Figs. 61C, 64C). Induction in cumin plants after treatment with 0.1% and 1.0% headline was noticed after 48 hours whereas in monitor treated plants induction of this activity was reported after 72 hrs.

In samples of third experimental plot experiment where two spray treatment was given with FOFCF, ABFCF and MIX FCF, maximum activity has been recorded with 10% FOFCF treatment in both GC-2 and GC-4 variety plants (Figs. 68C, 71C) on 6th day of the treatment giving 1.87 and 1.77 fold increase in β-1,3 glucanase
activity following 1st foliar treatment which is highest compared to all the other
treatments including control (Table 24) while in 2nd foliar spray, 1.68 fold with 10%
MIX FCF treatment in GC-2 variety plants on 6th day of the treatment and 1.51 fold
with 5% ABFCF treatment in GC-4 variety plants on 5th day of the treatment being
the highest activity (Table 25). The β-1,3 glucanase activity was observed higher in
all the treatments compared to control and remained high throughout the experiment.

Significant increased quantity of β-1,3 glucanase production was recorded by
Paul and Sarma (2005) in black pepper, when treated with Tricoderma isolates.
Nandini et al., (2010) reported the significant role of β-1,3 glucanase activity in
different varieties of groundnut. It was recorded higher in S. rolfsii, Aspergillus flavus
and A. parasiticus FCF treated plants at 96 hrs and then gradually decreased but
remain higher than the control plants throughout the experiment. Increased level of β-
1,3 glucanase activity indicates the SAR is induced in groundnut plants after the
treatments. Sriram et al. (2009) screened the Trichoderma harzianum elicitors for
their potential to induce systemic resistance against Phytophthora capsici in red
pepper plants. The effect of talc formulations on induction of β-1,3 glucanase activity
was significantly increased in red pepper plants compared to control. Leubner and
Meins (1999) have demonstrated that β-1, 3 glucanase was partially able to degrade
the cell wall and inhibit mycelial growth or spore germination of certain pathogenic
fungi. β-1,3 glucanase have not only the potential to hydrolyze cell components like
β-1,3 glucan, but they also release elicitors from the walls of fungi, which in turn
stimulate various defense responses in plants (Ren and West, 1992). Saikia et al.
(2005) demonstrated that Pseudomonas aeruginosa RsB29 induced systemic
resistance against Fusarium wilt of chickpea plants resulted in a significant increase
in PR-proteins, chitinases and β-1,3 glucanase. Singh et al. (1998) demonstrated the
application of Paenibacillus sp. 300 and Streptomyces sp. 385, which produce
chitinases and β-1,3 glucanases, provided excellent control of Fusarium wilt of
cucumber caused by F. oxysporum f. sp. cucumerinum in potting medium.

The results obtained on induction of β-1,3 glucanase activity in different
assays of the present study strongly supports the view of the induction of defense
response in both varieties of cumin plants. This induction may be due to the active
elicitor protein of 29 kDa of pathogenic FCFs used in the present study which is
supposed to be endochitinases of PR-3 family. This may have strong correlation in
inducing the $\beta$-1,3 glucanase enzyme in treated plants by the signaling molecules
generated upon treatment with the fungal FCF. As it was suggested that $\beta$ -glucanase
is expressed constitutively at low levels and is secreted into the cell wall and
intercellular spaces, where it encounters the invading fungus. An indirect defensive
role for $\beta$ -glucanase was suggested by the observation that $\beta$ -1,3/ 1,6-glucan
oligosaccharides termed oligosaccharide elicitors or oligosaccharins, which are
released from pathogen walls by the action of host glucanases can induce wide range
of plant defense responses (Ebel and Cosio, 1994). $\beta$-1,3 glucanase acts directly by
degrading the cell walls of the pathogen, and indirectly by promoting the release of
cell wall derived materials that can act as elicitors of defense reaction. Multiplicity of
$\beta$-1,3 glucanases functions might confer advantages to plants by providing several
lines of defense against invading microorganisms.

Moreover, foliar application of pathogenic fungal elicitors was seen to induce
defense response in cumin adequately compared to the other mode of application
selected. Thus foliar application can be the best mode of treatment in inducing SAR in
cumin. Further experiments were conducted at farmer’s field to ascertain the effect of
elicitors to combat the diseases.

**Polyphenol oxidase (PPO) activity:**

Polyphenol oxidase (PPO), a copper containing enzyme oxidizes phenolics to
highly toxic quinines and involved in the terminal oxidation of diseased plant tissues,
which was attributed for its role in disease resistance (Kosuge, 1969). These quinine
derivatives have antimicrobial activity. Because of its reaction products and wound
inducibility, PPO plays a role in defense against plant pathogen in plants (Chunhua et
al., 2001). Polyphenol oxidases (PPOs) are ubiquitous enzymes which use molecular
oxygen to oxidize common ortho-diphenolic compounds such as caffeic acid and
catechol to their respective quinones which is more toxic against pathogen and also
help in formation of lignin (Constabel and Barbehen, 2008).

Among the treatments the elicitor treatments have shown higher activity than
control plants and the activities varied at different time interval. The activity of PPO
was highest at 120 hrs in the seeds treated with FOFCF and *B. subtilis* CCF whereas
at 48 hrs with *T. harzianum* FCF respectively grown in soil infested with *F. oxysporum f. sp. cumini* and in the experimental plot of both the varieties (Figs. 32C, 34C). In *B. subtilis* CCF treated seeds grown in infested soil the highest fold count increase of PPO activity (Figs. 29C, 31C) was 2.07 in GC-2 and 1.76 in GC-4 whereas the PPO activity fold count was observed at 2.98 and 2.96 in GC-2 and GC-4 plants respectively growing in the experimental plot (Figs. 51C, 53C) (Table 18, 21).

In soil infestation experiment 3, elicitation of PPO activity with *F. oxysporum f. sp. cumini* infestation was higher at 1.60 fold in GC-2 plants with 10% FOFCF treatment at 120 hrs and 1.95 fold in GC-4 plants with 5% ABFCF treatment at 96 hrs (Figs. 37D, 40D) (Table 19). 5% ABFCF treated cumin seeds germinated in *A. burnsii* infested soil gave highest PPO activity of 2.45 and 2.26 fold increase at 96 hrs (Figs. 43D, 46D) in GC-2 and GC-4 varieties respectively (Table 20).

In first foliar spray assay in experimental plot, the highest PPO activity was observed at 120 hrs with FOFCF and *B. subtilis* CCF treatment while at 96 hrs with treatment of *T. harzianum* FCF (Figs. 56C, 58C). The highest increase in fold count of PPO activity i.e. 2.98 and 2.99 fold increase in GC-2 and GC-4 respectively was found in the treatment of FOFCF treatment (Table 22). The PPO activity remained high throughout the experiment compared with control plants in all the treatments.

In second experimental plot experiment, elicitation of PPO activity was higher at 2.64 fold in GC-2 variety and 1.63 fold in GC-4 variety (Table 23) with 10% FOFCF treated plants at 120 hrs. 0.1% headline and 0.5% monitor treated plants showed significant induction at 72 hrs and 48 hrs respectively which was lower than FOFCF treated plants but higher than untreated control plants (Figs. 61D, 64D).

In the third experimental plot experiment, 1st foliar spray showed higher PPO activity on 6th day of the treatment in GC-2 variety plants which was 1.68 fold with 10% FOFCF treatment and 1.84 fold in GC-4 variety with MIX FCF treatment on 6th day of the treatment (Table 24) but highest induction in activity was seen in plants treated with FOFCF on 6th day of the treatment in both varieties (Figs. 68D, 71D). On the other hand, in 2nd foliar spray, highest activity has been observed in ABFCF treated plants on 5th day of the treatment but highest fold induction resulted in MIX FCF treated plants with 1.85 fold in GC-2 variety plants on 6th day of the treatment.
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(Fig. 68D) and 5% ABFCF treatment showed 1.47 fold increases in GC-4 plants on 5th day of the treatment (Fig. 71D) (Table 25). Based on results obtained it can be concluded that treatment with FOFCF and ABFCF in 1st and 2nd foliar spray respectively enhanced the defense responses in cumin plants.

PPO is induced in response to mechanical wounding or response to elicitors in plants (Constabel et al., 2000). Systemic induction of PPO expression in response to wounding and pathogens might provide an additional line of defense to protect plants against further attack by pathogen and insects (Thipyapong et al., 1995). Tian et al. (2006) reported that the application of SA, calcium chloride and oxalic acid reduce the disease incidence in pear fruit caused by *Alternaria alternata* by inducing the activities of β-1, 3 glucanase, PAL, PPO and peroxidase. Raju et al. (2008) also noted higher levels of polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), β-1,3 glucanase (PR-2) and phenolics in roots and shoots of resistant cultivar than that of susceptible cultivar of chick pea upon treatment with elicitors and pathogen. Similarly elicitation of PPO activity was also found in *in vitro* propagated banana plants where 8.1 fold higher PPO activities after treatment with *Fusarium oxysporum f. sp. cubense* derived elicitors were observed by Patel et al. (2004). Three to four fold increase in the activity of PPO was observed by Nakkeeran et al., (2006) with *Pseudomonas chlororaphis* PA23 and *Bacillus subtilis* BSCBE4 in pre-treated hot pepper seedlings inoculated with *Pythium aphanidermatum*. Induction of PPO activity in susceptible and resistant variety was also studied in onion against *Alternaria palandui* infection after using *Pseudomonas florescence* based formulation (Karthikeyan et al., 2005). In this study also induction of enhanced PPO activity was observed in cumin plants treated with selected elicitors which confirms the induction of SAR. But, foliar application with selected pathogenic fungal culture filtrates induces SAR at the higher level compared to other treatments and mode of application. Hence, use of FOFCF and ABFCF could be more beneficial at the field level.

**Catalase activity:**

Catalase play a role of specific peroxidase and their function is to protect cells from toxic effects of H2O2 (Luhova et al., 2003). Catalase is similar to peroxidase and is implicated in the destruction of excess hydrogen peroxide generated by biotic and
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Abiotic stresses (Fernandez-Garcia et al., 2004). H$_2$O$_2$ is also involved in the induction of SAR. Because catalase converts H$_2$O$_2$ to water and oxygen and regulates H$_2$O$_2$ concentration in tissue (Magbanua et al., 2007), inhibition would result in accumulation of reactive oxygen species that may act as secondary messengers to induce SAR gene expression. Consistent with this idea inhibitors of catalase unrelated to SA, as well as H$_2$O$_2$ itself, can induce SAR gene expression. The pathogen derived elicitors and plant produced salicylic acid (SA) share some signal transduction components. Alternatively, inhibition of catalase may have direct effects on the reduction of pathogen growth (Ryals et al., 1994). Catalase activity generally induces to control or inhibit the over H$_2$O$_2$ secretion in plants infected with pathogens. Upon activation of SAR, means controlled accumulation of reactive oxygen species and induction of defense related activities, catalase is found to be inhibited (Lee et al., 1995; Ryals et al., 1995).

In experiment 1 of pot assay and FOFCF, T. harzianum FCF, B. subtilis CCF treated seeds grown in experimental plot and foliar application of these elicitors on cumin plants grown in experimental plots, catalase activity in the treated plants decreased as compared to control on the first two days and subsequently increase or become almost equal to control plants as indicated in the Figs. 33B, 35B, 52B, 54B, 57B, 59B (Tables 18, 21, 22).

In present study, catalase activity was found to increase in GC-2 plants with 10% FOFCF treatment by 1.08 fold (Fig. 38A) and in GC-4 plants 5% ABFCF treatment induced catalase activity by 1.30 fold growing in F. oxysporum infested soil (Fig. 41A) (Table 19). While plants growing in A. burnii infested soil, highest fold increase of 0.97 and 1.0 was recorded (Figs. 44A, 47A) with 10% FOFCF treatment in GC-2 and GC-4 variety plants respectively at 120hrs (Table 20) in 3rd experiment of pot assay.

Catalase activity was found to be maximum in plants in second experimental plot experiment with 10% FOFCF at 48 hrs after the foliar spray treatment. While headline and monitor treated plants could induce catalase enzyme activity at 24 hrs after treatment. The induction of catalase enzyme activity was measured in terms of fold count where maximum 2.9 fold and 2.1 fold in GC-2 and GC-4 variety
respectively was observed with 10% FOFCF treatment (Table 23) (Figs. 62A, 65A) and varied among other treatments.

In third experimental plot assay, after 1st foliar spray, maximum catalase activity was observed with 10% FOFCF treated plants than ABFCF and MIX FCF. Fold count of 1.21 and 0.99 increase of catalase activity in GC-2 and GC-4 variety respectively in 10% FOFCF treated plants on 6th day of the treatment (Table 24) while in 2nd foliar spray, enhanced catalase activity was recorded with 5% ABFCF in both the varieties on 5th day of the treatment giving 1.12 and 1.26 fold increase in GC-2 and GC-4 plants respectively (Table 25) and varied among other treatments (Figs. 69A, 72A). These results strongly suggest that treatment with FOFCF and ABFCF in 1st and 2nd foliar spray respectively would be best to boost defense response in cumin plants.

Catalase was normally induced by commencement of mycorrhizal infection *Glomus mosseae* in tobacco root. The induction was seen initially but reduced with the transient enhancement of free SA level (Bililou et al., 2000). Paul and Sarma (2005) observed that the activity of catalase was found to be increased on the 2nd day but then gradually diminished. They also recorded that non-bacterized plants generate more catalase than the bacterized plants. Similar results were seen in the present study too. The reason for catalase to decrease initially and lower induction in much depends on the level of H$_2$O$_2$. Upon elicitor treatment H$_2$O$_2$ level increases as a part of defense mechanism. Continuous elicitation may lead to higher induction of H$_2$O$_2$ in the cells which is hazardous. This process takes several hours to days. After crossing optimum level, catalase comes in action to degrade H$_2$O$_2$ and thus maintains the level. Here in the present study also, similar effect was seen that highlights the role of catalase. Moreover, foliar spray treatment of pathogenic fungal culture filtrates showed best results compared to other selected agents in all the assays.

**Nitrate Reductase activity (NR)**

Plants obtain their nitrogen requirements mainly from the soil as nitrates. The fate of most of this nitrate is to be incorporated into proteins and nucleotides. Nitrate is first reduced to nitrite, which is subsequently reduced to ammonia. Ammonia is then incorporated into the amino acids, glutamine/glutamate using the C-skeletons
Kaiser, (1999) reported that inhibition of mitochondrial respiration by Pyraclostrobin activates the alternative oxidative pathway (AOX) which decreases the cellular level of ATP while [H+] in the cytosol. Its increase results in an activation of NADH-Nitrate reductase (NR). Activation of NR results, transitorily, in an increase in the nitrite levels and may enhance plant growth when N assimilation is a level limiter. Yamasaki and Takahasi (1999) were also found an increase in the production of N₂ via NR. In addition to that the nitric oxide synthase (NOS) process, there is an alternative pathway of NO production in plants, NR product with NADH- nitrite as substrate and signals generated by NO can trigger plant’s defensive system. Singh et al. (2010) reported that the increased contents of chlorophyll, total non-structural carbohydrate and total nitrogen, as well as nitrate assimilation through the induction of nitrate reductase activity in isolated cucumber cotyledons after salicylic acid treatment.

The NR activity varied among various treatments at different time interval. Soil infestation with *F. oxysporum* in third experiment of pot assay resulted in 1.74 fold increase in nitrate reductase activity with 10% FOFCF treatment in GC-2 variety and 1.76 fold with 5% ABFCF treatment in GC-4 variety (Table 19) (Figs. 38B, 41B). While 5% ABFCF treatment showed highest increase in fold count of 1.56 and 1.57 fold (Table 20) nitrate reductase activity in the both the varieties sown in *A. burnsii* infested soil (Figs. 44B, 47B).

Second experiment of experimental plot where foliar spray treatment with 10% FOFCF increased activity of nitrate reductase enzyme was measured at 2.25 fold and 2.37 fold at 120 hrs (Figs. 62B, 65B) (Table 23) which was highest in GC-2 and GC-4 varieties respectively and varied among other treatments. Induction in cumin plants after treatment with 0.1% and 1.0% headline was noticed after 48 hours whereas in monitor treated plants the activity was induced after 72 hrs. NR activity was found to be induced higher after the treatment with selected elicitors than control but maximum induction was reported with FOFCF treatment.

In the foliar spray treatment of third experimental plot experiment, 10% FOFCF exhibited highest induction with 3.55 and 2.60 fold increase of nitrate reductase enzyme activity in GC-2 and GC-4 variety plants respectively after 1st spray treatment on 6th day
of the treatment (Table 24) compared to ABFCF and MIX FCF. While after 2nd foliar spray treatment with 5% ABFCF resulted in 1.98 fold increase in GC-2 plants on 5th day of the treatment and MIX FCF gave 2.90 fold increase on 6th day of the treatment (Table 25) in nitrate reductase activity in GC-4 plants (Figs. 69B, 72B).

Nitrate reductase (NR) was recently shown to play an important role during phytopathogenic interactions by providing substrates for the synthesis of nitric oxide (NO), a key signaling molecule for plant defense responses (Hong et al., 2007; Oliveira et al., 2010). The reduction of nitrite to NO by nitrate reductase (NR), which is an essential enzyme for nitrogen assimilation, is additionally thought to be an important source of NO in plants (Yamasaki and Sakihama, 2000). Nitrate reductase serves plants, algae, and fungi as a central point for integration of metabolism by governing flux of reduced nitrogen by several regulatory mechanisms (Wilbur et al., 1999). Regulation of nitrate reduction was studied in wheat leaves by Naik et al. (1982) and in rice by Hemalatha (2002). Oliveira et al., (2010) reported that nitrate reductase-dependent nitric oxide synthesis in the induction of defense response in Arabidopsis thaliana against Pseudomonas syringae. Xu et al. (2012) have found that fungal elicitor prepared from the cell walls of Aspergillus niger induces multiple responses of Hypericum perforatum cells, including nitric oxide (NO) generation, jasmonic acid (JA) biosynthesis and hypericin production. Similar results have been observed in the present study which explains the role of NO and nitrate reductase enzyme in induction of defense in cumin plants. The induced activity noted in all the treatments especially higher nitrate reductase activity in 10% FOFCF, 5% ABFCF, 0.1% headline and 0.5% monitor in the present study indicates the indirect synthesis of NO which probably triggers defense response. Similar results on induction of nitrate reductase activity were observed in the present study strongly supports the view of the induction of defense response in both varieties of cumin plants.

**Nitrite Reductase activity (NiR)**

In soil infestation study of 3rd experiment designed in pot assay, induction of nitrite reductase activity was observed in cumin plants treated with 10% FOFCF of F. oxysporum at 1.57 and 1.38 fold in GC-2 and GC-4 variety plants respectively at 120 hrs (Table 19) and grown in F. oxysporum infested soil (Figs. 38C, 41C). Increase in 1.46 and 1.50 fold in nitrite reductase activity was observed with 10% FOFCF
treatment (Table 20) in GC-2 and GC-4 variety plants grown in *A. burnsii* infested soil respectively at 120 hrs (Figs. 44C, 47C). The induced nitrite reductase activity was observed at 120hrs in both 10% FOFCF, MIX FCF and at 96hrs in 5% ABFCF treated plants. Plants with other treatments showed less induction compared to FOFCF and ABFCF treatments.

Induction of nitrite reductase activity was observed in cumin plants in second experimental plot experiments treated with 10% FOFCF as foliar spray at 2.04 fold and 2.06 fold higher in GC-2 and GC-4 variety respectively (Table 23) at 120 hrs and varied among other treatments used in experiment. The induction of enzyme activity with 10% FOFCF treated plants was highest compared to other treatments throughout the experimental study (Figs. 62C, 65C). Induction in cumin plants after treatment with 0.1% & 1.0% headline was noticed after 48 hours where as in monitor treated plants induction in NiR activity was reported after 72 hrs.

Similar results were also recorded in two foliar spray treatment with FOFCF, ABFCF and MIXC FCF in third experimental plot experiment with highest fold increase of 1.33 and 1.41 fold in GC-2 and GC-4 plants respectively (Figs. 69C, 72C) after 10% FOFCF treatment during 1st foliar spray on 6th day of the treatment (Table 24). While in the 2nd foliar spray treatment, 5% ABFCF on 5th day of the treatment showed 1.59 fold in GC-2 plants and MIX FCF with 1.25 fold increased activity in GC-4 on 6th day of the treatment (Figs. 69C, 72C) plants being the highest (Table 25) and varied among other treatment.

Mishra et al., (2010) reported the role of nitrite reductase in plants that synthesize NO enzymatically by nitric oxide synthase, nitrite reductase and also by non-enzymatically in plants. NO play a diverse role in plant system including plant growth, stomata movement, iron homeostasis, protection against biotic and abiotic stresses, senescence etc. Van Camp et al., (1998) showed the relationship between nitric oxide, SA and reactive oxygen species (ROS) in the activation of defense genes and/or induction of death of host cells are better described as a self-simplifying process during which redox signalling through nitric oxide and ROS is stimulated by SA. SA inhibits the formation of jasmonate, which also results in decrease in the formation of ethylene and less peroxidation of lipids. In present study increased
activity of nitrite reductase was observed which strongly supports the view of the induction of defense response in GC-2 and GC-4 varieties of cumin plants.

**Estimation of total proteins and protein profiling:**

Hunt and Ryals (1996) studied the induced defense in plants and explained that higher plants have developed different mechanisms to protect themselves from various biotic and abiotic stresses, including pathogen attacks, wounding and exposures to heavy metals, salinity, drought, cold, air pollutants and ultraviolet rays. It induced a group of novel protein that protecting the affected plants from further infection. Tiryaki and Tunaz (2004) suggested that the plants show their defense mechanism against pathogens and insects by higher accumulation of protein. Saikia et al. (2006) also found the role of riboflavin in induction of systemic resistance against the fusarium wilt diseases. It can be concluded that bioelicitors used in this study probably activates the natural defense response by inducing the higher total proteins and finally contributes to the plant defense mechanism.

In all experiments, the level of total proteins in treated plants varied among treatments at different time interval. The total proteins in plants treated with *T. harzianum* were higher at 48 hrs in pot and seed treatment experimental plot assays, while the protein content was higher at 96 hrs in foliar spray treatment in experimental plot assay (Figs. 33C, 52C, 57C, 32C, 54C, 59C). The highest fold count 2.04 in GC-2 and 1.77 in GC-4 was found in plants treated with *B. subtilis* CCF and growing in infested soil whereas in plants growing in experimental plots the fold count was 2.54 and 2.7 in GC-2 and GC-4 respectively (Table 18, 21). The total proteins fold count increased up to 3.8 and 3.5 folds in GC-2 and GC-4 respectively in plants sprayed with FOFCF (Table 22). In all the treatments the total proteins increased throughout the experiment compared to control.

In experiment 3 of pot assay, plants growing in soil infested with *F. oxysporum* showed increase in protein concentration after treatment with 10% FOFCF (Figs. 38D, 41D) at 1.99 and 1.71 fold after 120hrs in GC-2 and GC-4 variety respectively (Table 19). In plants treated with 5% ABFCF and growing in *A. burnsii* infested soil, highest protein concentration has been recorded (Figs. 44D, 47D) at fold increase of 1.79 and 1.87 at 96 hrs in GC-2 and GC-4 variety respectively (Table 20).
In 2\textsuperscript{nd} experiment, higher total protein concentration was observed at 3.18 fold in both varieties of cumin plants treated with 10\% FOFCF at 120 hrs (Figs. 62D, 65D) (Table 23) and varied among other treatments. FOFCF treated showed good vigour and development compared to other treatments. 0.1\% headline and 0.5\% monitor treated plants showed significant induction of total proteins at 72 hrs and 48 hrs respectively which was lower than FOFCF treated plants but higher than untreated control plants.

Likewise, in third experiment, plant samples collected from treated plants after 1\textsuperscript{st} spray with selected elicitors, 1.73 and 1.28 fold increase in protein concentration was recorded with 10\% FOFCF treatment on 6\textsuperscript{th} day of the treatment (Figs. 69D, 72D) (Table 24) while 1.51 and 1.14 fold increase in 5\% ABFCF treated plants on 5\textsuperscript{th} day of the treatment in GC-2 and GC-4 variety respectively. Foliar spray with MIX FCF resulted in 1.70 and 1.31 fold increase in protein content in GC-2 and GC-4 varieties respectively on 6\textsuperscript{th} day of the treatment (Table 24). Following 2\textsuperscript{nd} foliar spray, increase in protein concentration by 1.31 fold was observed for both 10\% FOFCF and MIX FCF treated GC-2 variety plants on 6\textsuperscript{th} day of the treatment (Fig. 69D), which is seen slightly higher than 5\% ABFCF treated plants (Table 25). While in GC-4 variety plants, significant increase in protein content has been observed by 2.03 fold with MIX FCF treatment on 6\textsuperscript{th} day of the treatment (Fig. 72D) (Table 25).

Increase in protein concentration was also found in banana leaves after the treatment of culture filtrate of \textit{Fusarium oxysporum f. sp. cubense} strain-1 (Companioni et al., 2006). Sarwar et al. (2011) reported the increased concentration of total protein in chickpea plants treated with salicylic acid and Bion. These proteins may be pathogenesis related proteins (PR-proteins) i.e. peroxidase, chitinases and β-1,3-glucanases that probably induce systemic resistance in chickpea against \textit{Ascochyta} blight. Similarly, in the present study, 29kDa purified elicitor protein from both selected pathogenic FCFs that belongs PR-2 family and supposed to be β-1,3-glucanase acts upon cumin plants to enhance their defense response against infection. Therefore, induction of total protein concentration in present study with elicitors may be correlated with the induction of PR proteins which is important in SAR mechanism. This can also be correlated with the phenylpropanoid pathway where induction of PR proteins and defense related enzymes induces upon the signal elicits
by pathogenic elicitors. In the present study, treatment with pathogenic fungal eliciters, induction in total protein level induces at the highest level which confirms the acceptability of elicitors as an induced defense response. The foliar spray with elicitors being the best mode of treatment as it provokes the defense related enzymes at highest level.

**Protein Profiling by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

In the present study, protein profiling through SDS-PAGE revealed the presence of protein bands between the molecular weight range of 14.3 kDa - 97.4 kDa but more number of induced bands were seen between the ranges of 20.1-43.0 kDa in both varieties of cumin in various treatments. FOFCF treated plant samples resulted in more induced and prominent protein bands than other treated samples in all experiments.

In the first experiment of pot assay, induction in protein bands were recorded after SDS-PAGE protein profiling of samples obtained from both varieties of plants grown in infested soil after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF. More prominent and additional bands were seen in the profiling from the samples treated with FOFCF and *B. subtilis* CCF compared to *T. harzianum* FCF and control (Figs. 36A-B).

Similarly, in soil infestation experiment 3 cumin seeds of GC-2 and GC-4 variety were treated with FOFCF, ABFCF and MIX FCF and grown in *F. oxysporum f. sp. cumini* and *A. burnsii* infested soil separately. In the leaf samples collected after 21 days onwards, induction of proteins were seen clearly in treated samples than control in both the varieties (49A-B, 50A-B). These induced bands supposed to be the PR proteins that confer immunity to the plants. All the treated plants have shown induction of PR proteins that confirms that pathogenic FCFs have strong impact on establishment of SAR in cumin plants.

In the 1st experiment performed in experimental plot, SDS-PAGE analysis of protein profiling of samples obtained from plants grown after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF revealed the
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induction of additional protein bands in both the varieties (55A-B). These induced bands were seen higher in case of FOFCF treatment compared to other treatments.

In the 1st foliar spray experiment conducted on GC-2 and GC-4 varieties plants of cumin also revealed the induction of protein bands which were noticed in SDS-PAGE analysis (60A-B). In both the varieties, FOFCF treated plants have shown highest numbers of induced protein bands compared to B. subtilis CCF, T. harzianum FCF and control treatments.

SDS-PAGE analysis of proteins from the plant samples treated with 0.1% and 1.0% headline, 0.5% and 1.0% monitor and 10% FOFCF in second experimental plot experiment shown the induction of protein bands. Additional bands were recorded in both GC-2 and GC-4 varieties after various elicitor treatments (67A-B). Highest induction in protein bands were noticed in the samples treated with 10% FOFCF.

Foliar spray treatment to experimental plot grown GC-2 and GC-4 variety cumin plants confer the induction of additional protein bands after the treatment with FOFCF, ABFCF and MIX FCF. Two foliar spray treatments given at different time interval to both the varieties of cumin plants, maximum induction was seen after the treatment with pathogenic FCFs than control plants (74 A-D). The results obtained in third experimental plot experiments strictly confirm the induction of SAR by using fungal elicitors in cumin plants.

Elicitor treated plant samples resulted in more induced and prominent bands than other treated samples. Induction of proteins was observed by SDS-PAGE analysis in chickpea plants after the treatment with Bion and challenged with Ascochyta rabie (Sarwar et al., 2011). Prachi et al. (2002) also reported the induced PR proteins in the range of 14-43 kDa in calli of Zingiber officinale upon culture filtrate treatment of F. oxysporum f. sp. zingiberi. Sindelarova and Sindelar (2005) reported the induced proteins in the intercellular fluid (ICF) and leaf tissue of the hypersensitive tobacco cultivar Xanthi-nc inoculated with Tobacco mosaic virus (TMV) and observed the ICF and cell proteins of infected leaves including PR-proteins at the molecular weights of 15-16 kDa (Group 1), 27-28 kDa (Group 3: chitinases) and 36–40 kDa (Group 2a: β-1,3-glucanases). Nazeem et al. (2008) have studied the induction of PR protein using the SDS-PAGE and found the two
additional bands in tolerant leaves of chilli plant on second day after infection. Jebakumar et al., (2001) studied the induction of PR proteins and defense related enzymes in black pepper due to inoculation with *Phytophthora capsici* and noted that in susceptible varieties there was no such marked increase in protein after infection with *Phytophthora capsici*. Nafie and Mazen (2008) noticed the changes in protein banding patterns of soybean leaves in response to BTH (benzothiadiazole) treatment and after challenging with *Phialophora gregata*. Results obtained in this study also show that elicitor treatment induces low molecular weight proteins which may be responsible for the defense activities in the cumin plant. These low molecular weight proteins are supposed to be the PR proteins from various families and group. They can be group I, ii and iii endochitinases of PR-2 and 3 families having molecular weight of about 30 kDa, β-1, 3 glucanases of PR-2 family (m.w. 25-35kDa), endoprotease of PR-7 family, peroxidases like proteins of PR-9 family, ribonucleases of PR-10 family, plant defensins of PR-12 family, etc. that helps cumin plants in providing defense against pathogenic fungal infection.

**Isozyme study by native PAGE**

In recent years, there has been an increasing appreciation of the fact that enzymes commonly exist in multiple molecular forms (isozymes) within the cells and helps in development and disease resistance. Isozymes are an expression of the differentiation of cells. The occurrence of isozymes among plants is widespread. Peroxidase isozymes, because of their common occurrence and the ease of their detection, have been investigated more than any other plant isozyme to date. It has a significant role in plant development (Scandalios, 1974).

Maximum induction in isozyme peroxidase activity was observed with 10% FOFCF and 0.1% headline treated plant samples in both varieties of cumin (Fig. 67C, D) in comparison with control. Treatments with elicitor treated plants showed significant activity of peroxidase and clear induced bands after the gel stained with staining solution. Time course of peroxidase induction and change of apoplastic protein patterns were revealed by native PAGE. The induction of peroxidase isozyme patterns in treated plants correlates the induction of SAR in cumin plants after treatment with FOFCF.
Induction of peroxidase isozyme activity was noticed by Roth et al. (2000) in tobacco, cucumber and tomato plants with the treatment of brassinosteroid containing extract of *Lychnis viscaria*. Aboshosha et al. (2008) checked resistance and susceptibility of sunflower to *Macrophomina phaseolina* infection by analyzing the protein and peroxidase isozymes. Gogoi et al., (2001) observed the induction of isozyme patterns in wheat plants to check resistance against karnal bunt caused by *Neovossia indica*. Rahnama and Ebrahimzadeh (2006) reported the induction of peroxidase activity in potato plants under salt stress condition. Udomprasert and Attathom (1991) have checked POX isozyme activity in tomato plants infected by tomato yellow leaf curl virus. Karthikeyan et al., (2005) have studied peroxidase isozyme activity during induction of resistance in host against the infection of leaf blight pathogen in onion. Liang et al., (2005) reported the induction of β-1,3 glucanase isoforms which boost the immunity against the pathogen in *Arachis hypogaea* upon inoculation with *Aspergillus flavus*. Ye et al., (1990) studied the isozyme pattern and cellular localization of peroxidase in relation to systemic acquired resistance of tobacco to blue mold and tobacco mosaic virus and induction of catalase isozyme was also checked on gel by staining enzyme activity. In tobacco, spinach, maize, and barley, the existence of a distinct catalase isozyme with enhanced peroxide activity was seen after elicitor treatment (Evelyn et al., 1989). Phenol oxidase activity was higher in resistant wheat cultivar ACC-8226 than in susceptible cultivar MP-845 in control sets and after inoculation of *Alternaria triticina* (Tyagi et al., 2000). Polyphenol oxidase isozyme activity was observed in tomato roots inoculated with *Glomus clarum* (Yakelin et al., 2001). Similarly in this study also successful induction of plant defense related enzymes such as PAL, POX, β-1,3 glucanase, PPO, NR, NiR, total protein along with peroxidase isozyme was observed after elicitor treatments. The induced isozymes too support the other enzymes in conferring the defense to the host plants. This clearly confirms the view that systemic acquired resistance was induced in cumin plants.

**Estimation of total phenols:**

The phenolic compounds as constituents of lignin may contribute to enhance the mechanical strength of the host cell wall and may also inhibit fungal growth as they are fungitoxic in nature. Phenolic compounds are among the most influential and
widely distributed secondary products in the plants. Such compounds govern disease resistance in many crop plants. Phenolic compounds can be formed in response to the ingress of pathogens and their appearance is considered as part of an active defense (Nicholson and Hammerschmidt, 1992). Evidence strongly suggests that esterification of phenols to cell-wall materials is a common theme in the expression of plant resistance and it has been suggested that cross linking of such phenylpropanoid esters leads to the formation of lignin-like polymers (Fry, 1987). Localized responses to bacterial or fungal attacks often result in structural alterations such as lignification, involving multiple matrix components that include callose, phenolics and hydroxyproline-rich proteins (McLusky et al., 1999). Dililitas et al., (2010) observed the change in level of phenol content upon infection of tobacco mosaic virus in peppers. The induction of phenols might be due to the activation of shikimic acid pathway, through which the aromatic amino acids phenylalanine and tyrosine are formed and channeled for the synthesis of phenolics (Karthikeyan et al., 2006). Phenolic compounds are widely distributed in plants and a large increase in phenolic synthesis in plants is observed after infection with plant pathogens or as a response to fungal elicitor (Alberto et al., 2009). Matern and Kneusel (1998) proposed that elicitor treatment or infection causes rapid membrane changes leading to decrease in cytoplasmic pH. This decrease would have the effect of activating the hydroxylase, catalyzing the formation of caffeoyl-CoA from 4-coumaroyl-CoA. Rapid synthesis of these phenolics and their polymerization in the cell wall is an important first line of defense against infection.

In the present study, the amount of total phenols also increased along with the induction of defense related enzymes. The total concentration of phenols remained higher in all the treated plants as compared to control. Highest induction of phenols was recorded with pathogenic FCFs foliar treatment. Induced bands of phenols were also observed after phenol profiling in treated plants.

Induction of total phenols increased in both the varieties at 96 hrs, 120 hrs and 144 hrs with treatment of T. harzianum, F. oxysporum f. sp. cumini FCFs and B. subtilis CCF respectively in plants growing in F. oxysporum f. sp. cumini infested soil (Figs. 33D, 35D) in experiment 1 of pot assay. The highest increase in fold count of
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Total phenols was 2.12 in GC-2 and 1.91 in GC-4 plants treated with *B. subtilis* CCF (Table 18).

In soil infestation experiment 3 of pot assay, cumin seeds treated with FOFCF and grown in *F. oxysporum* infested soil exhibited highest activity of 2.23 and 1.62 fold at 120 hrs in GC-2 and GC-4 variety (Table 19) respectively (Figs. 39A, 42A). On the contrary, cumin seeds grown in *A. burnsii* infested soil following 5% ABFCF treatment showed the fold count increase of 1.93 and 1.95 fold (Table 20) activity in GC-2 and GC-4 variety plants respectively (Figs. 45A, 48A).

The level of phenols increased at 144 hrs in seeds treated with *B. subtilis* CCF and FOFCF while at 120 hrs in seeds treated with *T. harzianum* FCF and growing in experimental plots (Figs. 52D, 54D). The level of phenols increased up to 2.47 and 3.16 folds in GC-2 and GC-4 plants respectively in *B. subtilis* CCF treated plants growing in experimental plots (Table 21).

In foliar spray application during experiment 1, the phenols level was highest at 120 hrs with FOFCF and *B. subtilis* CCF treatments and at 96 hrs with *T. harzianum* FCF treatment (Figs. 57D, 59D). 10% FOFCF foliar spray treated plants exhibited the increase in phenol level up to 2.64 folds in GC-2 and 3.18 folds in GC-4 as compared to control (Table 22). The level of total phenols increased in all the treatments, but varied, in this study compared to control plants.

In second experiment, the highest total phenol concentration was found in 10% FOFCF treated plants at 2.38 fold in GC-2 variety and 2.40 fold in GC-4 variety at 120 hrs and varied in other treatments (Figs. 63A, 66A) (Table 23). Induction of phenols in cumin plants after treatment with 0.1% and 1.0% headline was noticed after 48 hours where as in monitor treated plants the induction in this activity was reported after 72 hrs.

The result of 1st foliar spray treatment with 10% FOFCF in third experiment showed the highest induction of total phenol concentration at 2.85 in GC-2 and 2.09 fold in GC-4 variety on 6th day of the treatment compared to ABFCF and MIX FCF treatment. In 2nd foliar spray treatment with 5% ABFCF, 2.03 and 1.40 fold increase in phenol content was observed in GC-2 and GC-4 variety respectively on 5th day of
the treatment which being the highest among all the other treatments (Table 24 & 25) (Figs. 70A, 73A). The results obtained confirm the use of 10% FOFCF as a spraying agent in 1st spray and ABFCF during 2nd spray to achieve the best induction of total phenols in cumin plants.

Mandal et al., (2010) stated that phenolic acids are the main polyphenols made by plants. These compounds have diverse functions and are immensely important in plant-microbe interactions/symbiosis. Phenolic compounds act as signaling molecules in the initiation of legume-rhizobia symbioses, establishment of arbuscular mycorrhizal symbioses and can act as agents in plant defense. Flavonoids are a diverse class of polyphenolic compounds that have received considerable attention as signaling molecules involved in plant-microbe interactions compared to the more widely distributed, simple phenolic acids; hydroxybenzoic and hydroxycinnamic acids, which are both derived from the general phenylpropanoid pathway. Raju et al., (2008) reported the higher levels of phenolics in roots and shoots of resistant cultivar of chickpea than that of susceptible cultivar on treatment with elicitors and pathogen. Induction of defense proteins and accumulation of phenolics might have contributed to restrict the invasion of *F. oxysporum* f. sp. *ciceri*, in resistant cultivar. Chitra et al., (2006) reported that the application of biocontrol agent by foliar spray and seed treatment with *Alternaria alternata* significantly reduced the leaf blight incidence both under green house and field condition by inducing phenolic contents in groundnut plants. Estrada et al., (2009) concluded that bio-elicitor activates the natural defense response as measure by the increase in PAL, phenolic acid synthesis and chitinase, in a similar way as the infection by the living pathogen. Sriram et al., (2009) have screened *Trichoderma harzianum* have potential to induce systemic resistance against *Phytophthora capsici* in red pepper plants and observed the increase in phenol content in plants treated with *Trichoderma harzianum* compared to control. Induction in phenols was reported by Nandini and Mohan (2009) after foliar application of FCF prepared from *S. rolfsii* upon different varieties of groundnut. Mahapatra et al. (2015) have also recorded the induction in phenol level in maize plants after foliar spray treatment with *Aspergillus flavus*, *A. parasiticus* FCF compared to control. Similarly in the present study too, induction in the phenol content in both the varieties of cumin endorses the induction of defense. Phenols are the basic component in plant defense. Their induction naturally provides strong line of
defense to the plants. Generation of phenols in more amount after the treatment with pathogenic FCFs specifically strictly supports the onset of SAR in cumin plants.

**Phenol profiling**

Phenol profiling through HPTLC was done for four set of different experiments. In soil infestation experiment 1 of pot assay and two experimental plot assays where treatment to the seeds and plants growing in plots given by FOFCF, *T. harzianum* FCF, *B. subtilis* CCF and induction of phenolic compounds were checked. Induction of phenols was also checked for the foliar spray treated plants with FOFCF, 0.1% and 1.0% headline and 0.5% and 1.0% monitor in the experimental plot experiment 2.

The HPTLC analysis of the samples obtained from soil infestation experiment revealed the induction of induced phenols in both the varieties in all the treatments in comparison with untreated control plants. Based on the Rf values, among the various phenols induced in plants treated with the three culture filtrates selected for study and growing in infested soil during 1st soil infestation experiment. Nine common bands were visualized along with control and treated plants (Fig. 75A-B). Additionally 3 bands were observed in *F. oxysporum. f. sp. cumini* FCF, 2 bands in *B. subtilis* CCF and *T. harzianum* FCF treated plants which are common in both the varieties (Table 15, 16).

Seeds treated with culture filtrates and grown in the experimental plots, the induction of phenols varied in both the varieties to some extent. In all the treatments including control, 11 common bands were visualized and additionally 4 bands in *F. oxysporum. f. sp. cumini* FCF treatment, 3 bands in *T. harzianum* FCF treated plants and 5 bands in *B. subtilis* treated plants were visualized in GC-2 variety (Fig. 75C). 9 common bands were recorded along with control and additionally 6 and 5 bands in *F. oxysporum. f. sp. cumini* FCF and *B. subtilis* CCF treated plants respectively and 2 bands in *T. harzianum* FCF treated plants of GC-4 variety (Fig. 75D) (Table 15, 16).

In foliar spray treated plants with culture filtrates also revealed the induction of phenols which varied among the treatments compared with control. Total 12 common bands were visualized in all the treatments including control. Seven
additional bands were observed in *F. oxysporum f. sp. cumini* FCF treated plants and 3 bands were observed in *B. subtilis* CCF and *T. harzianum* FCF treated plants of GC-2 variety (Fig. 75E). 11 common bands were recorded and 9 additional bands were visualized in *F. oxysporum f. sp. cumini* FCF treated plants whereas 4 and 3 bands were visualized in *B. subtilis* CCF and *T. harzianum* FCF treated plants of GC-4 variety (Fig. 75F) (Table 15, 16).

Among all the treatments foliar spray treated plants with *F. oxysporum f. sp. cumini* FCF induced more bands followed by *B. subtilis* CCF treated plants in both the varieties. This confirms to select foliar spray treatment as the best mode of treatment to induce SAR in cumin plants. Some common and additional bands were identified which are likely to occur specific phenols after comparing the Rf values of the phenols with that of standards which are mentioned in tables 8 and 9. Hydroxyquinone was found to be common in both GC-2 and GC-4 varieties. Coumaric acid with the Rf value 0.53 was found common in experimental plots assay in all the treatments but was found to be induced in soil infestation study after the treatment with *B. subtilis* CCF in both the varieties. Caffeic acid was absent in soil infestation study but found to be induced in elicitor treated seeds and plants grown in experimental plots in both the varieties. Salicylic acid was found to be induced in all experiments but only after the treatment with the *Fusarium oxysporum f. sp. cumini* FCF. This result supports the theory of SAR which is established via SA as signaling molecule. Interesting result was noticed in foliar spray experiment that the unidentified phenol with the Rf value 0.71 was found induced only in GC-4 variety after treatment with FOFCF and was not observed in any other treatment. This particular phenol is supposed to be the treatment and variety specific phenol. Several other common and induced but unidentified phenols were also recorded which also helps in providing the defense to the plants.
Table 15: Rf values of different phenols in the control and treated plants of GC-2 variety.

<table>
<thead>
<tr>
<th>Phenols likely to happen in relation to the Rf values</th>
<th>C</th>
<th>F</th>
<th>B</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyquinone</td>
<td>0.49</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Coumaric acid</td>
<td>0.53</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.61</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Catechol</td>
<td>0.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferulic acid</td>
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<td>Galic acid</td>
<td>1.04</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>1.08</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>1.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trans-chlorogenic acid</td>
<td>1.11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rf values

<table>
<thead>
<tr>
<th>Pot Assay</th>
<th>Seed Treatment Field Assay</th>
<th>Foliar Spray Treatment Field Assay</th>
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</thead>
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<td>0.66</td>
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<td>0.93</td>
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<td>No. of Common bands visualized</td>
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<td>9</td>
</tr>
<tr>
<td>No. of induced bands visualized</td>
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<td>3</td>
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C- Control, F- *F. oxysporum f. sp. Cumini* FCF, B- *B. subtilis* CCF and T- *T. harzianum* FCF treatments
Table 16: Rf values of different phenols in the control and treated plants of GC-4 variety.

<table>
<thead>
<tr>
<th>Phenols likely to happen in relation to the Rf values</th>
<th>Pot Assay</th>
<th>Seed Treatment Field Assay</th>
<th>Foliar Spray Treatment Field Assay</th>
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<tr>
<td></td>
<td>C</td>
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<td>B</td>
</tr>
<tr>
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<td>0.15</td>
<td>0.15</td>
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<td>-</td>
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<tr>
<td></td>
<td>0.44</td>
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<tr>
<td></td>
<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>Standards Phenols</td>
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</tr>
<tr>
<td>Hydroxyquinone</td>
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</tr>
<tr>
<td>Coumaric acid</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Caffeic acid</td>
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</tr>
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<td>Ferulic acid</td>
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<tr>
<td>Gallic acid</td>
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</tr>
<tr>
<td>Salicylic acid</td>
<td>1.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trans-chlorogenic acid</td>
<td>1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total No. of bands visualized</td>
<td>9</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>No. of Common bands visualized</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>No. of induced bands visualized</td>
<td>nil</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

C- Control, F- *F. oxysporum f. sp. cumini* FCF, B- *B. subtilis* CCF and T- *T. harzianum* FCF treatments
In the experiment 3 of pot assay, HPTLC analysis revealed induction of phenolic compounds in elicitors treated plants. 0.1% and 1.0% headline treated plants showed 3 induced bands, while 0.5% and 1.0% monitor treatment have 1 and 2 induced bands respectively. Maximum induction was seen with 10% FOFCF treated plants where 4 induced bands appeared in GC-2 variety (Fig. 76A-B). In GC-4 variety also 6 maximum numbers of induced bands were observed in the sample treated with 10% FOFCF.

In this experiment, phenols were identified by comparing the Rf values of samples with standard phenols Rf values. In GC-2 variety, hydroxyquinone and cinnamic acid were commonly present in all treated and untreated plants. Salicylic acid and caffeic acid was found to be induced with 10% FOFCF treatment in cumin plants of GC-2 variety (Table 17). While hydroxyquinone, ferulic acid, cinnamic acid and galic acid were found to be common in all treated and untreated plants of GC-4 variety of cumin. Salicylic acid, vanillic acid and trans-chlorogenic acid were found to be induced in 10% FOFCF treated GC-4 plants (Tables 17).

Plant phenolics are secondary metabolites that encompass several classes structurally diverse of natural products biogenetically arising from the shikimate-phenylpropanoids-flavonoids pathways. Plant needs phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. Phenols are known to be antimicrobial for many plants. It functions as precursor molecule for lignin synthesis (Lattanzio et al., 2006). Mandavia et al., (2000) reported the exhibition of higher content of phenolic compounds such as salicylic acid and hydroquinone in tolerant cumin plants which inhibited the spore formation and mycelia growth of *F. oxysporum* thereby controlling the wilt disease in cumin.

In this study caffeic acid was found to be induced in GC-2 variety only. Salicylic acid being the only phenol which was found to be induced in both GC-2 and GC-4 varieties after 10% FOFCF treatment only. This strongly suggests that SAR has established in cumin via SA as a signaling molecule and hence these phenols, phytoalexins and defense related enzymes are getting produced at the induced level. Just like SA, vanillic acid and trans-chlorogenic acid were also found as induced phenols in GC-4 variety only after 10% FOFCF treatment. The variety and treatment specific phenols may have significant role in defense mechanism of plants.
Table 17: Presence of identified phenols in treated and untreated plants

<table>
<thead>
<tr>
<th>Standard phenols</th>
<th>Rf values of standard phenol</th>
<th>Colour of bands after vanillin sulphuric acid spray</th>
<th>Treatments in GC-2 variety</th>
<th>Treatment in GC-4 variety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>0.1% H</td>
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<tr>
<td>Hydroxyquinone</td>
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<td>Purple</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>0.49</td>
<td>Violet</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Coumaric acid</td>
<td>0.53</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>0.61</td>
<td>Green</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Catechol</td>
<td>0.74</td>
<td>Purple</td>
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<td>-</td>
</tr>
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<td>Ferulic acid</td>
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</tr>
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<td>Galic acid</td>
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<td>Salicylic acid</td>
<td>1.08</td>
<td>purple</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vanillic acid</td>
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<td>Pink</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Trans-chlorogenic acid</td>
<td>1.11</td>
<td>Gray</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Reducing sugar concentration

Carbohydrates are the basic building blocks for the synthesis of various defense chemicals such as phenolics, phytoalexins and lignin. Hence the quality and quantity of sugars play an important role in disease resistance. Induction of carbohydrate metabolism in relation to leaf blight (*Bipolaris sorokiniana*) was studied in healthy and infected leaves of barley (*Hordeum vulgare*) by Singh et al. (2009) where sugar has played significant role in disease resistance. The results of both reducing and non-reducing sugars in this study indicate that the plants are preparing for the enhanced metabolic activities for induction of various defense related enzymes.

In soil infestation study of pot assay experiment 3, seeds treated with elicitors and grown on soil infested with *Fusarium oxysporum f. sp. cumini*, 1.84 fold increase in reducing sugar content in GC-2 variety plants was observed with 5% ABFCF treatment at 96 hrs (Fig. 39B) and with 10% FOFCF treatment 2.11 fold increase was recorded at 120 hrs (Fig. 42B) in GC-4 plants (Table 19). On the other hand, plants treated with 5% ABFCF and growing in *Alternaria burnsi* infested soil showed marked increase in reducing sugar concentration observed at 2.72 and 2.45 fold in GC-2 and GC-4 variety at 96 hrs respectively (Figs. 45B, 48B) (Table 20).

In 2nd experimental plot experiment, 10% FOFCF treated plants resulted in induction of 2.72 fold increase in reducing sugar content in GC-2 variety and 2.55 fold in GC-4 variety (Table 23) at 120 hrs and varied at different time interval (Figs. 63B, 66B). Induction in cumin plants after treatment with 0.1% and 1.0% headline was noticed after 48 hours where as in monitor treated plants induction in this activity was reported after 72 hrs.

Reducing sugar content was also induced in plants growing in third experimental plot experiment after 1st foliar spray after the treatment with FOFCF, ABFCF and MIX FCF. Treatment with 10% FOFCF in 1st foliar application resulted in highest induction at 2.69 and 1.84 fold increase in GC-2 and GC-4 variety respectively on 6th day of the treatment (Figs. 70B, 73B) (Table 24). While in 2nd foliar spray, enhanced sugar induction was recorded at 1.94 fold in GC-2 (Fig. 70B) and 2.66 fold in GC-4 variety (Fig. 73B) on 5th day of the treatment with 5% ABFCF (Table 25).
Non reducing sugar concentration

The concentration of non-reducing sugar was induced in treated plants with elicitors at 120 hrs and grown in both *F. oxysporum* and *A. burnsii* infested soils in experiment 3 of pot assay. Increase in non-reducing sugar 2.21 and 2.34 fold was recorded in GC-2 and GC-4 variety respectively in plants growing in *F. oxysporum* infested soil after the seeds treated with 10% FOFCF (Table 19) (Figs. 39C, 42C). Similarly, the plants growing in soil infested with *A. burnsii* (Figs. 45C, 48C) after seed treatment with 10% FOFCF, 2.12 and 2.10 increase in fold count was observed in GC-2 and GC-4 variety plants at 120 hrs respectively (Table 20).

The highest non-reducing sugar concentration was found in 10% FOFCF treated plants growing in second experimental plot experiment with 2.69 fold in GC-2 and 2.67 fold in GC-4 cumin variety at 120 hrs (Table 23) (Figs. 63C, 66C) and varied among other treatments. Induction in cumin plants after treatment with 0.1% and 1.0% headline was recorded after 48 hours whereas in monitor treated plants the induction in this activity was seen after 72 hrs.

Foliar application with FOFCF, ABFCF and MIX FCF, 10% FOFCF treated plants in third experimental plot experiment after 1st foliar spray showed highest increase in non-reducing sugar content, 5.35 fold, on 6th day of the treatment in GC-2 variety and 1.68 fold in GC-4 variety plants (Table 24). Second spray treatment with 5% ABFCF plants showed 2.46 and 2.97 fold increase in non-reducing sugar content on 5th day of the treatment in GC-2 and GC-4 varieties respectively (Table 25) which was found to be highest among all the other treatments (Figs. 70C, 73C). The results obtained revealed that the use of FOFCF during 1st foliar application and ABFCF during 2nd foliar application to boost the immunity in cumin plants. This mode of treatment should be applied to field level for the benefit of farmers.

Tadeje et al., (1998) described the role of sugar in plants. Sugar metabolism plays a significant role during the defense response by increasing sugar contents. When plants are infected with avirulent pathogen, selected groups of plant cell rapidly die by hypersensitive response. It leads to expression of defense response and increased the induction of sugar content up to 10-12 folds at infected site. Rolland et al., (2002) have shown the interaction of sugar and protein molecules during plant pathogen interaction to form the step of signaling module. Essmann et al., (2008)
reported the rapid metabolism of carbohydrate is an important factor determining the outcome of plant pathogen interaction. Liebenberg (2007) have checked the sugar signaling events during the infection of wheat with *Puccinia triticina*. In the present study, cumin plant response with elicitor treatment has elevated the concentration of reducing and non-reducing sugars throughout the experiment which may be correlated with the signaling process upon activation of defense response in plants.

In the present study similar results are obtained which confirms the role of SAR in disease resistance in cumin plants. Moreover, all selected bioelicitors have shown positive impact in triggering SAR but two spray FOFCF and ABFCF treatment resulted in highest and noteworthy effect on controlling the diseases compared to headline, monitor, *T. harzianum* FCF and *B. subtilis* CCF. Additionally, pathogenic fungal elicitors are easy to prepare and apply over the plants. They are environment friendly and cost effective. Among all the mode of treatment used in the present study, foliar application is the best suitable mode to provoke SAR in cumin plants. Thereby, FOFCF and ABFCF can be used as two spray treatment in field study.

Table 18: Highest fold count recorded in samples obtained from the 25 days old plants growing in *F. oxysporum f. sp. cumini* infested soil after the seed treatment in both the varieties.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Fold count of Enzyme activities</th>
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</thead>
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<tr>
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<td></td>
<td>PAL</td>
</tr>
<tr>
<td>GC-2</td>
<td><em>F. oxysporum f. sp. cumini</em></td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
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</tr>
<tr>
<td></td>
<td><em>T. harzianum</em></td>
<td>1.48</td>
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<td>2.06</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>2.19</td>
</tr>
<tr>
<td></td>
<td><em>T. harzianum</em></td>
<td>1.44</td>
</tr>
</tbody>
</table>
Table 19: Fold count of estimated defense related enzymes in GC-2 and GC-4 variety plants grown in *Fusarium oxysporum f. sp. cumini* infested soil after FOFCF, ABFCF and MIX FCF treatment.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Induction Time of highest activity (hrs)</th>
<th>PAL</th>
<th>POX</th>
<th>β-1,3 glucanase</th>
<th>PPO</th>
<th>Catalase</th>
<th>NR</th>
<th>NIR</th>
<th>Total Protein</th>
<th>Total Phenol</th>
<th>Reducing Sugar</th>
<th>Non-Reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td>10% FOFCF</td>
<td>120</td>
<td>1.93</td>
<td>2.10</td>
<td>2.58</td>
<td>1.60</td>
<td>1.08</td>
<td>1.74</td>
<td>1.57</td>
<td>1.99</td>
<td>2.23</td>
<td>1.71</td>
<td>2.21</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>96</td>
<td>1.81</td>
<td>1.95</td>
<td>2.21</td>
<td>1.32</td>
<td>1.05</td>
<td>1.32</td>
<td>1.18</td>
<td>1.80</td>
<td>1.98</td>
<td>1.84</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>120</td>
<td>1.74</td>
<td>1.99</td>
<td>2.53</td>
<td>1.44</td>
<td>0.90</td>
<td>1.73</td>
<td>1.42</td>
<td>1.84</td>
<td>1.95</td>
<td>1.73</td>
<td>1.97</td>
</tr>
<tr>
<td>GC-4</td>
<td>10% FOFCF</td>
<td>120</td>
<td>1.93</td>
<td>2.04</td>
<td>2.50</td>
<td>1.62</td>
<td>1.26</td>
<td>1.61</td>
<td>1.38</td>
<td>1.71</td>
<td>1.62</td>
<td>2.11</td>
<td>2.34</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>96</td>
<td>1.69</td>
<td>1.89</td>
<td>2.13</td>
<td>1.95</td>
<td>1.30</td>
<td>1.76</td>
<td>1.32</td>
<td>1.75</td>
<td>1.56</td>
<td>1.95</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>120</td>
<td>1.76</td>
<td>1.97</td>
<td>2.48</td>
<td>1.56</td>
<td>1.21</td>
<td>1.59</td>
<td>1.27</td>
<td>1.62</td>
<td>1.55</td>
<td>1.93</td>
<td>2.15</td>
</tr>
</tbody>
</table>
Table 20: Fold count of estimated defense related enzymes in GC-2 and GC-4 variety plants grown in *Alternaria burnsi* infested soil after FOFCF, ABFCF and MIX FCF treatment.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Induction Time of highest activity (hrs)</th>
<th>PAL</th>
<th>POX</th>
<th>β-1,3 glucanase</th>
<th>PPO</th>
<th>Catalase</th>
<th>NR</th>
<th>NIR</th>
<th>Total Protein</th>
<th>Total Phenol</th>
<th>Reducing Sugar</th>
<th>Non-Reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td>10% FOFCF</td>
<td>120</td>
<td>2.64</td>
<td>2.74</td>
<td>2.16</td>
<td>1.51</td>
<td>0.97</td>
<td>1.42</td>
<td>1.46</td>
<td>1.51</td>
<td>1.59</td>
<td>2.31</td>
<td>2.12</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>96</td>
<td>2.55</td>
<td>2.10</td>
<td>2.68</td>
<td>2.45</td>
<td>0.96</td>
<td>1.56</td>
<td>1.23</td>
<td>1.79</td>
<td>1.93</td>
<td>2.72</td>
<td>1.65</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>120</td>
<td>2.54</td>
<td>2.74</td>
<td>2.08</td>
<td>1.46</td>
<td>0.96</td>
<td>1.38</td>
<td>1.35</td>
<td>1.50</td>
<td>1.57</td>
<td>2.13</td>
<td>1.97</td>
</tr>
<tr>
<td>GC-4</td>
<td>10% FOFCF</td>
<td>120</td>
<td>2.51</td>
<td>2.60</td>
<td>2.72</td>
<td>1.50</td>
<td>1.0</td>
<td>1.49</td>
<td>1.50</td>
<td>1.60</td>
<td>1.73</td>
<td>2.37</td>
<td>2.10</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>96</td>
<td>2.10</td>
<td>1.79</td>
<td>2.59</td>
<td>2.26</td>
<td>0.93</td>
<td>1.57</td>
<td>1.26</td>
<td>1.87</td>
<td>1.95</td>
<td>2.45</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>120</td>
<td>2.30</td>
<td>2.56</td>
<td>2.58</td>
<td>1.39</td>
<td>1.0</td>
<td>1.48</td>
<td>1.34</td>
<td>1.57</td>
<td>1.53</td>
<td>2.33</td>
<td>2.03</td>
</tr>
</tbody>
</table>
Table 21: Highest fold count recorded in samples collected from 25 day old experimental plot grown plants after seed treatment with culture filtrates in both the varieties of cumin.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>PAL</th>
<th>POX</th>
<th>PPO</th>
<th>β-1,3 glucanase</th>
<th>Proteins</th>
<th>Phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td><em>F. oxysporum f. sp. cumini</em></td>
<td>1.60</td>
<td>1.82</td>
<td>2.91</td>
<td>2.63</td>
<td>2.47</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>1.69</td>
<td>2.38</td>
<td>2.98</td>
<td>2.68</td>
<td>2.54</td>
<td>2.47</td>
</tr>
<tr>
<td></td>
<td><em>T. harzianum</em></td>
<td>1.55</td>
<td>2.12</td>
<td>2.11</td>
<td>2.52</td>
<td>2.40</td>
<td>2.30</td>
</tr>
<tr>
<td>GC-4</td>
<td><em>F. oxysporum f. sp. cumini</em></td>
<td>1.55</td>
<td>1.75</td>
<td>2.94</td>
<td>2.70</td>
<td>2.35</td>
<td>3.09</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>1.71</td>
<td>1.756</td>
<td>2.96</td>
<td>2.98</td>
<td>2.70</td>
<td>3.16</td>
</tr>
<tr>
<td></td>
<td><em>T. harzianum</em></td>
<td>1.68</td>
<td>1.750</td>
<td>2.53</td>
<td>2.61</td>
<td>2.33</td>
<td>2.46</td>
</tr>
</tbody>
</table>

Table 22: Highest fold count recorded in samples collected from 25 day old field grown plants after foliar spray treatment in both the varieties.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>PAL</th>
<th>POX</th>
<th>PPO</th>
<th>β-1,3 glucanase</th>
<th>Proteins</th>
<th>Phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td><em>F. oxysporum f. sp. cumini</em></td>
<td>2.05</td>
<td>2.17</td>
<td>2.98</td>
<td>3.23</td>
<td>3.80</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>1.97</td>
<td>1.76</td>
<td>2.69</td>
<td>2.38</td>
<td>3.17</td>
<td>2.54</td>
</tr>
<tr>
<td></td>
<td><em>T. harzianum</em></td>
<td>1.94</td>
<td>1.31</td>
<td>1.78</td>
<td>1.91</td>
<td>3.17</td>
<td>1.92</td>
</tr>
<tr>
<td>GC-4</td>
<td><em>F. oxysporum f. sp. cumini</em></td>
<td>2.23</td>
<td>2.88</td>
<td>2.99</td>
<td>3.44</td>
<td>3.50</td>
<td>3.18</td>
</tr>
<tr>
<td></td>
<td><em>B. subtilis</em></td>
<td>1.95</td>
<td>2.63</td>
<td>2.02</td>
<td>2.72</td>
<td>3.32</td>
<td>2.20</td>
</tr>
<tr>
<td></td>
<td><em>T. harzianum</em></td>
<td>1.78</td>
<td>2.40</td>
<td>1.86</td>
<td>2.02</td>
<td>2.4</td>
<td>1.98</td>
</tr>
</tbody>
</table>
Table 23: Highest fold count of various enzymes and biochemical parameters recorded in second experimental plot assay from 25 day old field grown plants of GC-2 and GC-4 variety after foliar spray treatment with Headline, Monitor and FOFCF.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatments</th>
<th>PAL</th>
<th>POX</th>
<th>β-1,3 glucanase</th>
<th>PPO</th>
<th>Catalase</th>
<th>Nitrate Reductase</th>
<th>Nitrite Reductase</th>
<th>Total Protein</th>
<th>Total Phenol</th>
<th>Reducing Sugar</th>
<th>Non reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td>0.1% Headline</td>
<td>2.4</td>
<td>3.39</td>
<td>2.30</td>
<td>1.45</td>
<td>2.3</td>
<td>1.84</td>
<td>1.85</td>
<td>2.10</td>
<td>1.87</td>
<td>2.54</td>
<td>2.51</td>
</tr>
<tr>
<td></td>
<td>1.0% Headline</td>
<td>2.71</td>
<td>3.39</td>
<td>2.32</td>
<td>1.91</td>
<td>1.41</td>
<td>2.45</td>
<td>2.50</td>
<td>2.84</td>
<td>2.60</td>
<td>1.77</td>
<td>1.76</td>
</tr>
<tr>
<td></td>
<td>0.5% Monitor</td>
<td>2.51</td>
<td>3.30</td>
<td>2.26</td>
<td>1.24</td>
<td>1.26</td>
<td>2.25</td>
<td>2.19</td>
<td>2.06</td>
<td>1.81</td>
<td>1.86</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td>1.0% Monitor</td>
<td>2.3</td>
<td>3.31</td>
<td>2.12</td>
<td>1.30</td>
<td>2.0</td>
<td>1.57</td>
<td>1.75</td>
<td>1.38</td>
<td>1.33</td>
<td>2.36</td>
<td>2.29</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>3.25</td>
<td>4.21</td>
<td>2.87</td>
<td>2.64</td>
<td>2.9</td>
<td>2.25</td>
<td>2.04</td>
<td>3.18</td>
<td>2.38</td>
<td>2.72</td>
<td>2.69</td>
</tr>
<tr>
<td>GC-4</td>
<td>0.1% Headline</td>
<td>2.73</td>
<td>4.02</td>
<td>4.01</td>
<td>1.63</td>
<td>1.9</td>
<td>1.95</td>
<td>1.85</td>
<td>2.93</td>
<td>2.36</td>
<td>2.74</td>
<td>2.58</td>
</tr>
<tr>
<td></td>
<td>1.0% Headline</td>
<td>2.51</td>
<td>3.63</td>
<td>3.94</td>
<td>1.64</td>
<td>1.25</td>
<td>1.76</td>
<td>2.56</td>
<td>2.84</td>
<td>2.22</td>
<td>2.56</td>
<td>2.51</td>
</tr>
<tr>
<td></td>
<td>0.5% Monitor</td>
<td>2.39</td>
<td>3.30</td>
<td>2.30</td>
<td>1.36</td>
<td>1.58</td>
<td>1.68</td>
<td>2.16</td>
<td>2.06</td>
<td>1.84</td>
<td>2.16</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>1.0% Monitor</td>
<td>2.42</td>
<td>3.89</td>
<td>2.49</td>
<td>1.26</td>
<td>1.5</td>
<td>1.90</td>
<td>1.75</td>
<td>2.15</td>
<td>1.90</td>
<td>2.34</td>
<td>2.53</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>3.28</td>
<td>4.21</td>
<td>4.03</td>
<td>1.63</td>
<td>2.1</td>
<td>2.37</td>
<td>2.06</td>
<td>3.18</td>
<td>2.40</td>
<td>2.55</td>
<td>2.67</td>
</tr>
</tbody>
</table>
Table 24: Fold count of estimated defense related enzymes in GC-2 and GC-4 variety plants grown in experimental plot after 1st foliar spray treatment given to 21 days germinated cumin plants with FOFCF, ABFCF and MIX FCF.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Induction day</th>
<th>PAL</th>
<th>POX</th>
<th>β-1,3 glucanase</th>
<th>PPO</th>
<th>Catalase</th>
<th>NR</th>
<th>NIR</th>
<th>Total Protein</th>
<th>Total Phenol</th>
<th>Reducing Sugar</th>
<th>Non-Reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td>10% FOFCF</td>
<td>$6^{th}$</td>
<td>1.65</td>
<td>1.50</td>
<td>1.87</td>
<td>1.68</td>
<td>1.21</td>
<td>3.55</td>
<td>1.33</td>
<td>1.73</td>
<td>2.85</td>
<td>2.69</td>
<td>5.35</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>$5^{th}$</td>
<td>1.75</td>
<td>1.48</td>
<td>1.33</td>
<td>1.59</td>
<td>1.20</td>
<td>2.54</td>
<td>1.15</td>
<td>1.51</td>
<td>2.71</td>
<td>1.53</td>
<td>2.48</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>$6^{th}$</td>
<td>1.36</td>
<td>1.42</td>
<td>1.77</td>
<td>1.47</td>
<td>1.21</td>
<td>3.48</td>
<td>1.27</td>
<td>1.70</td>
<td>2.86</td>
<td>2.38</td>
<td>5.17</td>
</tr>
<tr>
<td>GC-4</td>
<td>10% FOFCF</td>
<td>$6^{th}$</td>
<td>2.22</td>
<td>1.38</td>
<td>1.77</td>
<td>1.76</td>
<td>0.99</td>
<td>2.60</td>
<td>1.41</td>
<td>1.28</td>
<td>2.09</td>
<td>1.84</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>$5^{th}$</td>
<td>1.50</td>
<td>1.28</td>
<td>1.60</td>
<td>1.35</td>
<td>0.98</td>
<td>2.36</td>
<td>1.30</td>
<td>1.14</td>
<td>1.80</td>
<td>1.84</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>$6^{th}$</td>
<td>1.80</td>
<td>1.21</td>
<td>1.69</td>
<td>1.84</td>
<td>0.97</td>
<td>2.50</td>
<td>1.32</td>
<td>1.31</td>
<td>2.01</td>
<td>1.83</td>
<td>1.57</td>
</tr>
</tbody>
</table>
Table 25: Fold count of estimated defense related enzymes in GC-2 and GC-4 variety plants grown in third experimental plot after 2

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Induction day</th>
<th>PAL</th>
<th>POX</th>
<th>β-1,3 glucanase</th>
<th>PPO</th>
<th>Catalase</th>
<th>NR</th>
<th>NIR</th>
<th>Total Protein</th>
<th>Total Phenol</th>
<th>Reducing Sugar</th>
<th>Non-Reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td>10% FOFCF</td>
<td>6\textsuperscript{th}</td>
<td>1.80</td>
<td>1.35</td>
<td>1.63</td>
<td>1.84</td>
<td>1.11</td>
<td>1.85</td>
<td>1.31</td>
<td>1.31</td>
<td>1.87</td>
<td>1.84</td>
<td>1.75</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>5\textsuperscript{th}</td>
<td>1.75</td>
<td>1.65</td>
<td>1.57</td>
<td>1.39</td>
<td>1.12</td>
<td>1.98</td>
<td>1.59</td>
<td>1.30</td>
<td>2.03</td>
<td>1.94</td>
<td>2.46</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>6\textsuperscript{th}</td>
<td>1.89</td>
<td>1.34</td>
<td>1.68</td>
<td>1.85</td>
<td>1.02</td>
<td>1.72</td>
<td>1.26</td>
<td>1.31</td>
<td>1.84</td>
<td>1.76</td>
<td>1.72</td>
</tr>
<tr>
<td>GC-4</td>
<td>10% FOFCF</td>
<td>6\textsuperscript{th}</td>
<td>1.71</td>
<td>1.32</td>
<td>1.29</td>
<td>1.21</td>
<td>1.25</td>
<td>2.79</td>
<td>1.24</td>
<td>1.93</td>
<td>1.69</td>
<td>1.58</td>
<td>1.86</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>5\textsuperscript{th}</td>
<td>1.48</td>
<td>1.50</td>
<td>1.51</td>
<td>1.47</td>
<td>1.26</td>
<td>1.76</td>
<td>1.17</td>
<td>1.56</td>
<td>1.40</td>
<td>2.66</td>
<td>2.97</td>
</tr>
<tr>
<td></td>
<td>MIX FCF</td>
<td>6\textsuperscript{th}</td>
<td>1.79</td>
<td>1.39</td>
<td>1.35</td>
<td>1.22</td>
<td>1.20</td>
<td>2.90</td>
<td>1.25</td>
<td>2.03</td>
<td>1.69</td>
<td>1.70</td>
<td>1.84</td>
</tr>
</tbody>
</table>
Farmer’s field experiment:

Based on the positive results obtained with pathogenic fungal elicitors in soil infestation and experimental plots experiments, use of fungal elicitors as foliar application may be the best option to combat wilt and blight diseases of cumin. After the preliminary experiments, field experiments were conducted to know the efficacy of pathogenic fungal elicitors at field level following the normal agricultural practices at farmer’s field. Foliar application being the best mode of treatment, FOFCF and ABFCF along with headline as positive control was applied to elicit the SAR in cumin plants. Many researchers have performed such experiments to know the effect of fungal elicitors for their use at large scale in combating disease spread. Mahapatra et al., (2015) noticed induction of various enzymes associated with defense after foliar treatment with FCF of *Aspergillus flavus* and *A. parasiticus* on field grown maize plants of Pioneer- 30V92 cv variety. They reported good yield and significant defense induction against ear rot disease caused by fungus. Amin et al., (2015) evaluated the ability of Headline (Pyraclostrobin 20% WG) to induce systemic resistance in 40-day-old field-grown groundnut plants by foliar application and observed elevated levels of defense-related enzymes such as phenylalanine ammonia lyase, peroxidase, β 1,3 glucanase, nitrate and nitrite reductase and other biochemical parameters such as total phenol, total protein, total carbohydrate and total chlorophyll content were found highest in Headline treated plants on fourth day after treatment in comparison with untreated plants. Chitra et al., (2008) have also reported the induction of chitinase, glucanase and PAL in field grown 32 days old peanut plants against disease caused due to the infection of *Alternaria alternata* after the treatment of SA. Similarly, in the present study too, induction in defense response was seen. Disease spread was restricted and better yield as well as vigour was observed. After the application of fungal elicitors, induction in phenylpropanoids was recorded. FOFCF and ABFCF containing the signaling peptides provoke the formation of defense related enzymes in cumin plants. This is in correlation of the mechanism of phenylpropanoid pathway where signaling molecule be the reason of developing SAR in plants. Eventually developed SAR helps plants to survive against disease and more yield can be obtained which helps farmer to fetch the benefit. Moreover, use of such biological elicitor is harmless to human and animal. It is cost effective as well as environmental friendly.
In the 1st farmer’s field experiment conducted in the year 2011-12, foliar spray treatment to cumin plants grown in two different fields of GC-2 and GC-4 variety respectively at Shantipura village of Sabarkantha district, Gujarat were given with 10% FOFCF, 0.1 and 1.0% headline. Plants treated with normal water were used as control.

Foliar spray treatment with selected elicitors given to both the varieties of cumin plants resulted in induction of all defense related enzymes, total protein, total phenol, reducing and non reducing sugar after the treatment with 0.1% and 1.0% headline at 48 hrs and 10% FOFCF at 120 hrs but varied among treatments at different time interval (Table 26). Highest induction of all activities was seen after treatment with 10% FOFCF. The highest fold activity of PAL was recorded with 10% FOFCF i.e. 2.84 fold with GC-2 variety (Fig. 77A) and 3.29 fold in GC-4 variety (Fig. 80A). POX activity was seen to be induced at the rate of 5.86 fold and 5.75 fold in GC-2 variety (Fig. 77B) and GC-4 variety (Fig. 80B) respectively. β-1,3 glucanase enzyme showed highest induction of 1.91 fold in treated GC-2 variety (Fig. 77C) and 1.56 fold in treated GC-4 variety (Fig. 80C) of cumin. Induction of PPO activity was observed at 1.44 and 3.03 fold in GC-2 variety (Fig. 77D) and GC-4 variety (Fig. 80D) treated plants respectively with 10% FOFCF. Catalase activity was also induced higher in both varieties treated plants (Figs. 78A, 81A) in FOFCF treated plants. Induction in terms of fold count in nitrate reductase activity was 1.51 fold and 0.98 fold and nitrite reductase activity was 1.82 fold and 2.11 fold in GC-2 and GC-4 variety plants treated with 10% FOFCF (Fig. 78B-C; 81B-C) (Table 26).

Induction in total protein concentration in 10% FOFCF treated plants at 3.13 fold and 3.18 fold higher in GC-2 and GC-4 cumin varieties respectively (Fig. 78D, 81D). Induction in total phenol concentration at 2.77 fold in GC-2 variety (Fig. 79A) and 2.65 fold in GC-4 variety (Fig. 82A) was seen after treatment with 10% FOFCF. Induction in reducing sugar concentration at 3.0 fold in both GC-2 (Fig. 79B) and GC-4 variety plants (Fig. 82B) whereas induction in non reducing sugar concentration at 2.46 fold with GC-2 variety (Fig. 79C) and 2.50 fold with GC-4 variety (Fig. 82C) cumin variety plants observed after treatment with 10% FOFCF (Table 26). Highest induction of enzyme activities were observed in FOFCF treated
plants compared to Headline and untreated control plants. All the enzyme activities were remained higher throughout the experiment in treated plants with elicitors than control plants.

Table 26: Highest fold count of different enzymes and biochemical parameters recorded in 25 days old field grown cumin plants at Shantipura village after treatment with elicitors.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatments</th>
<th>PAL</th>
<th>POX</th>
<th>β-1,3 glucanase</th>
<th>PPO</th>
<th>Catalase</th>
<th>Nitrate Reductase</th>
<th>Nitrite Reductase</th>
<th>Total Protein</th>
<th>Total Phenol</th>
<th>Reducing Sugar</th>
<th>Non reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td>0.1% Headline</td>
<td>2.28</td>
<td>2.55</td>
<td>1.82</td>
<td>1.06</td>
<td>0.8</td>
<td>1.38</td>
<td>1.54</td>
<td>2.95</td>
<td>1.20</td>
<td>2.40</td>
<td>2.37</td>
</tr>
<tr>
<td></td>
<td>1.0% Headline</td>
<td>2.10</td>
<td>2.4</td>
<td>1.80</td>
<td>1.44</td>
<td>1.04</td>
<td>1.49</td>
<td>1.36</td>
<td>2.83</td>
<td>2.68</td>
<td>2.25</td>
<td>2.51</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>2.84</td>
<td>5.86</td>
<td>1.91</td>
<td>1.44</td>
<td>1.0</td>
<td>1.51</td>
<td>1.82</td>
<td>3.13</td>
<td>2.77</td>
<td>3.00</td>
<td>2.46</td>
</tr>
<tr>
<td>GC-4</td>
<td>0.1% Headline</td>
<td>2.86</td>
<td>5.11</td>
<td>1.26</td>
<td>1.01</td>
<td>0.9</td>
<td>1.60</td>
<td>1.05</td>
<td>2.98</td>
<td>2.83</td>
<td>2.54</td>
<td>2.48</td>
</tr>
<tr>
<td></td>
<td>1.0% Headline</td>
<td>2.66</td>
<td>4.63</td>
<td>2.26</td>
<td>1.66</td>
<td>1.25</td>
<td>2.11</td>
<td>1.88</td>
<td>2.84</td>
<td>2.67</td>
<td>2.38</td>
<td>2.35</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>3.29</td>
<td>5.75</td>
<td>1.56</td>
<td>3.03</td>
<td>1.2</td>
<td>0.98</td>
<td>2.11</td>
<td>3.18</td>
<td>2.65</td>
<td>3.00</td>
<td>2.50</td>
</tr>
</tbody>
</table>

In second field experiment conducted at Sarla village, Ahmedabad district in the year 2012-13, two foliar spray treatments were given to the GC-2 variety cumin plants. In 1st foliar application, treatment was given with 0.1% headline and 10% FOFCF to 25 days old plants while in 2nd spray treatment 5% ABFCF was used in lieu of 10% FOFCF and given to 55 days old plants. Highest induction of various defense related enzyme activities were noted after 1st foliar application in field grown cumin plants after the treatment with 10% FOFCF than 0.1% headline and control plants. Higher fold activity (Table 27) of various enzymes such as PAL (2.56) (Fig. 84A), POX (1.56) (Fig. 84B), β-1, 3 glucanase (2.47) (Fig. 84C), PPO (1.46) (Fig. 84D), catalase (1.18) (Fig. 85A), nitrate reductase (3.36) (Fig. 85B), nitrite reductase (3.81) (Fig. 85C) was recorded in plants treated with FOFCF on the 6th day after treatment. Highest induction in fold count of total protein (1.14) (Fig. 86A), total phenol (3.27) (Fig. 86B), reducing sugar (3.87) (Fig. 86C) and non reducing sugar (3.05) (Fig. 86D) content was noticed after 1st foliar application with 10% FOFCF on 6th day after treatment than 0.1% headline treated and untreated control plants (Table 27).
Induction in all the enzymatic activities after 0.1% headline treatment was noticed but the activities were less than 10% FOFCF treatment and more than control.

Noteworthy induction of various enzymes was also observed after the 2nd spray with 5% ABFCF on 5th day after treatment. Highest fold induced activity (Table 27) of various enzymes viz. PAL (1.61) (Fig. 84A), POX (1.85) (Fig. 84B), β-1,3 glucanase (2.78) (Fig. 84C), PPO (1.20) (Fig. 84D), catalase (1.10) (Fig. 85A), nitrate reductase (2.61) (Fig. 85B), nitrite reductase (2.39) (Fig. 85C) was seen in plants treated with 5% ABFCF. Total protein content (2.44) (Fig. 86A), total phenol (1.62) (Fig. 86B), reducing sugar (2.60) (Fig. 86C) and non reducing sugar (1.33) (Fig. 67D) contents were increased on 5th day after treatment in plants treated with 5% ABFCF than 0.1% headline and control plants.
Table 27: Fold induction of various defense related enzymes in control and treated plants with 10% FOFCF & 0.1% Headline in 1\textsuperscript{st} spray to 25 days old cumin plants and 5 % ABFCF & 0.1% Headline in 2\textsuperscript{nd} spray to 55 days old plants in the 2\textsuperscript{nd} field experiment conducted at Sarla village, Ahmedabad district.

<table>
<thead>
<tr>
<th>Spray</th>
<th>Treatment</th>
<th>Time of Induction of highest activity</th>
<th>PAL</th>
<th>POX</th>
<th>β-1,3 glucanase</th>
<th>PPO</th>
<th>Catalase</th>
<th>NRA</th>
<th>NIR</th>
<th>Total Protein</th>
<th>Total Phenol</th>
<th>Reducing Sugar</th>
<th>Non-Reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{st}</td>
<td>10% FOFCF</td>
<td>6\textsuperscript{th} day</td>
<td>2.56</td>
<td>1.56</td>
<td>2.47</td>
<td>1.46</td>
<td>1.18</td>
<td>3.36</td>
<td>3.81</td>
<td>1.14</td>
<td>3.27</td>
<td>3.87</td>
<td>3.05</td>
</tr>
<tr>
<td></td>
<td>0.1% Headline</td>
<td>3\textsuperscript{rd} day</td>
<td>1.67</td>
<td>1.14</td>
<td>1.95</td>
<td>1.12</td>
<td>1.04</td>
<td>1.69</td>
<td>2.12</td>
<td>1.25</td>
<td>2.51</td>
<td>1.46</td>
<td>1.97</td>
</tr>
<tr>
<td>2\textsuperscript{nd}</td>
<td>5% ABFCF</td>
<td>5\textsuperscript{th} day</td>
<td>1.61</td>
<td>1.85</td>
<td>2.78</td>
<td>1.20</td>
<td>1.10</td>
<td>2.61</td>
<td>2.39</td>
<td>2.44</td>
<td>1.62</td>
<td>2.60</td>
<td>1.33</td>
</tr>
<tr>
<td></td>
<td>0.1% Headline</td>
<td>3\textsuperscript{rd} day</td>
<td>1.39</td>
<td>1.36</td>
<td>2.56</td>
<td>1.19</td>
<td>1.05</td>
<td>2.46</td>
<td>2.00</td>
<td>1.72</td>
<td>1.53</td>
<td>1.75</td>
<td>1.26</td>
</tr>
</tbody>
</table>
The third field experiment was conducted in farmer’s field at Sarla village of Ahmedabad district in the year 2013-14. After 25 days of sowing the seeds, 1st foliar spray was given to four sets of plants separately i.e. with D/W (control), 10% FOFCF, 5% ABFCF and 0.1% Headline. Samples were collected and processed at different time interval for estimation of different enzyme activities. Induction of defense related enzyme activities were found to be more in fungal elicitor treated plant samples than headline treated or control plants. Moreover, FOFCF treated plant samples showed highest activities and biochemical parameters at 120 hours after treatment compared to all other treatments in both sprays (Table 28).

At different time interval, higher fold enzyme activities (Table 28) viz. PAL (2.59) (Fig. 88A), POX (1.67) (Fig. 88B), β- 1, 3 glucanase (2.56) (Fig. 88C), PPO (1.63) (Fig. 88D), catalase (1.23) (Fig. 89A), nitrate reductase (2.92) (Fig. 89B), nitrite reductase (3.18) (Fig. 89C) were recorded in plants treated with FOFCF. Highest induction in fold of total protein (1.24) (Fig. 90A), total phenol (2.94) (Fig. 90B), reducing sugar (2.79) (Fig. 90C) and non-reducing sugar (2.60) (Fig. 90D) content was reported after 1st foliar application with 10% FOFCF at 120 hrs than 0.1% headline and 5% ABFCF treated and untreated control plants. However, higher activities of all enzymes were noticed in plants treated with elicitors than control throughout the experiment (Table 28).

Noteworthy change in enzymatic activities was also seen after the 2nd spray with fungal elicitors and Headline. 10% FOFCF treated plants showed highest enzymatic activities than other treatments throughout the experiment (Table 29). Plants treated with 5% ABFCF and AB(FO) FCF also has showed good induction of defense related enzyme activities compared to 0.1% Headline treated plants at different time interval. Highest fold induced enzyme activities (Table 22) viz. PAL (2.33) (Fig. 91A), POX (2.47) (Fig. 91B), β- 1, 3 glucanase (3.68) (Fig. 91C), PPO (2.05) (Fig. 91D), catalase (1.21) (Fig. 92A), nitrate reductase (2.85) (Fig. 92B), nitrite reductase (3.43) (Fig. 92C) were seen in plants treated with 10% FOFCF. Highest induction in fold count of total protein content (3.46) (Fig. 93A), total phenol (1.85) (Fig. 93B), reducing sugar (2.25) (Fig. 93C) and non reducing sugar (1.36) (Fig. 93D) content was observed at 120 hrs in FOFCF treated plants than 0.1% headline treated and control plants. The results obtained strictly corroborate the
conclusion made from the results of the studies performed at the lab scale and experimental plot. The results obtained in this assay also draw the attention towards the efficacy of FOFCF to induce SAR parameters during first foliar application and ABFCF during second foliar spray treatment.

These results can also be correlated with the specific disease incidence in cumin plants. Generally, wilt occurs during the earlier stage of the growth (Tawfik and Allam, 2004a) and blight occurs during or after flowering stage (Gemavat and Prasad, 1971). Besides this, *Fusarium oxysporum* can cause disease to the plant at any stage of growth and development. In case of blight disease, *Alternaria burnsii* targets the plants during or after flowering as sucrose and maltose - good carbon source are available during this stage. But treatment with fungal elicitors prior to the disease incidence stage may enhance the immunity and make the plants ready to cope up with the pathogens. Because, upon elicitation by elicitors, several defense related enzymes and physical barriers gets activated. Enzymes related to defense which were activated in advance acts directly on pathogenic organisms and restrict their spread via rupturing the cell wall and fundamental molecules. Upon infection, plants can speedily recognizes the pathogens as they were previously came in contact via deactivated pathogenic content present in elicitors during treatment. This will in turn speed up the process of signaling and secretion of defense related components. Whereas physical barriers activated in advance also help plants by restricting the entry of the pathogens.

Plants treated with fungal elicitors appeared healthy and there was no incidence of wilt and blight disease whereas disease symptoms in control plants observed throughout the experiment. Defense related enzymes have specific role in controlling the pathogenic entry in plants as well as they also actively boost the growth and development of the plant which in proportion lead to obtain higher yield. Moreover, the application of elicitors resulted in highest induction of defense related enzymes than other chemical agents including biochemical elicitors. Thus, use of FOFCF as 1st foliar application and ABFCF during 2nd foliar spray may be considered as one of the best practice of treatment to control cumin plants via SAR against the disease.
Table 28: Fold induction of various defense related enzymes in control and treated plants of GC-2 variety after 1st spray with elicitors (10% FOFCF, 5% ABFCF & 0.1% Headline) in the 3rd field experiment conducted at Sarla village, Ahmedabad district.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Induction Time of enzyme activities (Hrs)</th>
<th>PAL</th>
<th>POX</th>
<th>β-1,3 glucanase</th>
<th>PPO</th>
<th>Catalase</th>
<th>NRA</th>
<th>NIR</th>
<th>Total Protein</th>
<th>Total Phenol</th>
<th>Reducing Sugar</th>
<th>Non-Reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td>0.1% Headline</td>
<td>48</td>
<td>1.73</td>
<td>1.22</td>
<td>1.74</td>
<td>1.16</td>
<td>1.04</td>
<td>2.04</td>
<td>2.22</td>
<td>1.21</td>
<td>2.80</td>
<td>1.55</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>120</td>
<td>2.59</td>
<td>1.67</td>
<td>2.56</td>
<td>1.63</td>
<td>1.23</td>
<td>2.92</td>
<td>3.18</td>
<td>1.24</td>
<td>2.94</td>
<td>2.79</td>
<td>2.60</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>96</td>
<td>2.10</td>
<td>1.50</td>
<td>1.81</td>
<td>1.62</td>
<td>1.21</td>
<td>2.37</td>
<td>2.88</td>
<td>1.23</td>
<td>2.87</td>
<td>2.02</td>
<td>2.42</td>
</tr>
</tbody>
</table>
Table 29: Fold induction of various defense related enzymes in control and treated cumin plants of GC-2 variety after 2nd spray with elicitors in the 3rd field experiment conducted at Sarla village, Ahmedabad district.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Induction Time of enzymes (Hr)</th>
<th>1st 2nd</th>
<th>PAL</th>
<th>POX</th>
<th>β-1,3 glucanase</th>
<th>PPO</th>
<th>Catalase</th>
<th>NRA</th>
<th>NIR</th>
<th>Total Protein</th>
<th>Total Phenol</th>
<th>Reducing Sugar</th>
<th>Non-Reducing Sugar</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC-2</td>
<td>0.1% H</td>
<td>48</td>
<td>1.50 1.49</td>
<td>0.55</td>
<td>1.36</td>
<td>1.12</td>
<td>2.35</td>
<td>2.11</td>
<td>1.70</td>
<td>1.08</td>
<td>1.70</td>
<td>1.20</td>
<td>1.48</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>120</td>
<td>1.53 1.59</td>
<td>1.64</td>
<td>1.09</td>
<td>0.94</td>
<td>1.83</td>
<td>2.04</td>
<td>2.24</td>
<td>1.30</td>
<td>1.48</td>
<td>1.15</td>
<td>1.98</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>96</td>
<td>1.07 1.47</td>
<td>1.42</td>
<td>1.26</td>
<td>1.10</td>
<td>2.18</td>
<td>2.14</td>
<td>1.62</td>
<td>1.28</td>
<td>1.98</td>
<td>1.16</td>
<td>2.25</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>120</td>
<td>2.33 2.47</td>
<td>3.68</td>
<td>2.05</td>
<td>1.21</td>
<td>2.85</td>
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<td>3.46</td>
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<td>1.36</td>
<td>2.17</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>96</td>
<td>1.59 1.87</td>
<td>2.33</td>
<td>1.93</td>
<td>1.16</td>
<td>2.71</td>
<td>2.38</td>
<td>2.29</td>
<td>1.59</td>
<td>2.17</td>
<td>1.24</td>
<td>2.16</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td>5% ABFCF</td>
<td>96</td>
<td>1.60 1.95</td>
<td>2.31</td>
<td>1.89</td>
<td>1.16</td>
<td>2.66</td>
<td>2.35</td>
<td>2.27</td>
<td>1.56</td>
<td>2.16</td>
<td>1.20</td>
<td>2.02</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>10% FOFCF</td>
<td>120</td>
<td>2.17 2.13</td>
<td>3.27</td>
<td>1.86</td>
<td>1.15</td>
<td>2.60</td>
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<td>1.64</td>
<td>2.02</td>
<td>1.26</td>
<td>2.02</td>
<td>1.26</td>
</tr>
</tbody>
</table>
SDS-PAGE Protein Profiling

In the 2nd field experiment conducted at Sarla village, Ahmedabad district during 2012-13, SDS-PAGE analysis for proteins revealed the induction of protein bands in the samples collected from treated plants during both sprays. Moreover, fungal elicitor treated plants showed induction of some common and prominent additional bands compared to headline treated and control plant samples (Fig. 87A). Maximum number of induced bands was observed in the range of 66 - 43 kDa molecular weight.

Results of SDS-PAGE analysis performed with the samples collected from 3rd field experiment conducted at Sarla village, Ahmedabad district during 2013-14 shown that fungal elicitor treated plants showed induction of some additional protein bands along with common protein bands compared to headline treated and control plants (Fig. 94A-B) in both sprays. Among different treatments, plants treated with FOFCF showed comparatively more number of induced protein bands. This clearly indicates that FOFCF is more effective for induction of PR proteins. Induced bands were observed in the range of 66-20.1 kDa molecular weight.

These induced bands along with the common bands helps cumin plants in providing strong line of defense against the source of infection in the field. These proteins are supposed to be the low molecular weight PR proteins such as endochitinases, endo glucanases, defensin like proteins, protease inhibitors, peroxidases like proteins etc. which acts directly by inhibiting the growth of pathogens by shattering cell wall or indirectly on the plants by providing supplemental compounds that helps in boosting the immune response. Moreover, these results also supports the observations made in the soil infestation and experimental plot experiments carried out earlier and drawn the conclusion from those results to give the application of fungal elicitors at the large scale in field.

**Physiological parameters:**

Induction in resistance in plants upon bioelicitors treatments eventually leads to better growth and yield reported by several workers. Moreover, less disease incidence, more vigour and better growth of the plants naturally generate best yield.
Farouk and Osman (2011) upon treatment with various concentrations of elicitors reported significantly improved common bean plant growth i.e. positive effect on plant height, number of branches, shoot dry weight and leaf area per plant and bean yield. Thakur and Sohal (2014) concluded that elicitors act synergistically to promote growth and metabolic activities in *B. juncea* and *B. napus* cultivars leading to the induction and regulation of disease resistance upon their field experiment as they seen maximum contents of total soluble protein, free amino acids, total sugars and reducing sugars than control in all studied cultivars. They found that elicitor treatments were more pronounced in increasing plant height, internodal distance and number of pods per plant.

In the first field experiment conducted at Shantipura village, Sabarkantha district in the year 2011-12, the treated and untreated cumin plants were collected to evaluate the physiological parameters like height of the plants and number of umbels. Both variety cumin plants treated with 10% FOFCF resulted in increased height and more number of umbels compared to other treatments including control (Table 30) (Fig. 83 A-F).

Table 30: Effects on growth parameters in elicitor treated and untreated cumin plants (2011-2012)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Height of plants</th>
<th>No. of umbels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GC-2</td>
<td>GC-4</td>
</tr>
<tr>
<td>Control</td>
<td>21.75±3.97</td>
<td>18.05±4.49</td>
</tr>
<tr>
<td>10% FOFCF</td>
<td>41.13±5.93</td>
<td>34.37±6.04</td>
</tr>
<tr>
<td>0.1% headline</td>
<td>34.70±5.54</td>
<td>27.3±3.49</td>
</tr>
<tr>
<td>1.0% headline</td>
<td>25.96±7.75</td>
<td>23.03±5.07</td>
</tr>
</tbody>
</table>

In the second field experiment conducted at Sarla village of Ahmedabad district in the year 2012-13, marked difference in vigour was observed in fungal elicitor and 0.1% headline treated plants compared to untreated control plants growing in the field (Fig. 87 B-C). All selected physiological parameters height of the plant, number of the umbels, branches per plant, number of seeds per plant, weight of 100...
seeds and weight of seeds per gram were recorded highest in fungal elicitors treated plants compared to headline treated and untreated plants as shown in table 31. The reason behind better growth and development of the cumin plants may be the positive effect of fungal elicitors. These elicitors provoke the defense related as well as growth supportive enzymes and hormones in plants. Due to which higher vigour and yield is obtained. In the present study, cumin plants treated with fungal elicitors appeared healthy and there was no incidence of wilt and blight disease observed throughout the experiment. Moreover, disease incidence was noticed in control plants but not appeared in the fungal treated plants (Fig. 31E-G).

Table 31: Effects on physiological parameters of elicitors treated and untreated cumin plants (2012-2013)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Height of the plants (cm)</th>
<th>Number of branches per plant</th>
<th>Number of umbels per plant</th>
<th>Number of seeds per plant</th>
<th>Weight of 100 seeds (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.88±5.00</td>
<td>23.52±9.62</td>
<td>25.36±6.97</td>
<td>309±111.58</td>
<td>0.338±0.04</td>
</tr>
<tr>
<td>Fungal elicitors</td>
<td><strong>52.80±4.54</strong></td>
<td><strong>52.80±8.63</strong></td>
<td><strong>50.32±7.27</strong></td>
<td><strong>1281.04±386.49</strong></td>
<td><strong>0.477±0.03</strong></td>
</tr>
<tr>
<td>Headline</td>
<td>49.54±4.45</td>
<td>36.28±10.69</td>
<td>34.76±7.61</td>
<td>914.32±258.30</td>
<td>0.383±0.05</td>
</tr>
</tbody>
</table>

In the 3rd field experiment conducted at the Sarla village of the Ahmedabad district during the year 2013-14 positive results were obtained after the treatment with fungal elicitors. The details of physiological parameters noted in table 32, which showed noteworthy changes in the height of the plant, branches per plant, number of the umbels, number of seeds per plant and weight of 100 in elicitor treated plants compared to control at the time of harvest (Fig. 95A-C).
Table 32: Effects on physiological parameters of elicitors treated and untreated cumin plants (Sample size: 25 plants for each treatment) (2013-14)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Height of the plants (cm)</th>
<th>Number of branches per plant</th>
<th>Number of seeds per plant</th>
<th>Number of umbels per plant</th>
<th>Weight of 100 seeds (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.72±4.70</td>
<td>26.8±6.39</td>
<td>561.56±138.24</td>
<td>32.84±7.08</td>
<td>0.368±0.03</td>
</tr>
<tr>
<td>0.1% Headline</td>
<td>40.92±4.48</td>
<td>40.24±8.50</td>
<td>963.64±229.81</td>
<td>40.28±7.87</td>
<td>0.381±0.05</td>
</tr>
<tr>
<td>0.1% Headline (FO)</td>
<td>45.36±4.96</td>
<td>44.68±6.75</td>
<td>1222.68±288.83</td>
<td>48.2±7.02</td>
<td>0.494±0.03</td>
</tr>
<tr>
<td>0.1% Headline (AB)</td>
<td>42.52±5.47</td>
<td>42.8±9.20</td>
<td>1095.4±239.88</td>
<td>41.76±6.60</td>
<td>0.438±0.05</td>
</tr>
<tr>
<td>FOFCF</td>
<td>53.88±5.81</td>
<td>55.16±10.26</td>
<td>1405.28±315.08</td>
<td>57.56±8.64</td>
<td>0.634±0.09</td>
</tr>
<tr>
<td>FO (AB) FCF</td>
<td>48.48±13.61</td>
<td>50.24±8.49</td>
<td>1319.4±257.14</td>
<td>52.72±6.25</td>
<td>0.522±0.12</td>
</tr>
<tr>
<td>ABFCF</td>
<td>46.48±9.43</td>
<td>46.4±10.07</td>
<td>1223.92±204.02</td>
<td>50.2±5.52</td>
<td>0.510±0.09</td>
</tr>
<tr>
<td>AB (FO) FCF</td>
<td>49.88±3.72</td>
<td>52.92±7.39</td>
<td>1381.56±273.22</td>
<td>54.52±7.27</td>
<td>0.563±0.10</td>
</tr>
</tbody>
</table>

Treatment with FOFCF was very effective compared to all other treatments either alone or in combination (Table 32). FOFCF treated plants showed more induction of biochemical as well as physiological parameters compared to other treatments. Marked difference in greening and vigour was also observed in fungal elicitor and headline treated plants compared to untreated control plants growing in field (Fig. 95A-C). These results suggest that fungal elicitor has the effective role in inducing the growth and development of the plants. These results also support the onset of SAR in cumin plants after the application of fungal elicitors.

Positive physiological effects such as high carbohydrate content, protein content, chlorophyll content and proper nitrogen assimilation leads to better yield in crop plants (Taiz and Zeiger, 2010). In this study, the increase in yield in elicitor treated plants may be correlated with the stimulation of physiological processes especially higher protein synthesis, carbohydrate content and NR and NiR activities upon elicitor treatments. These in turn facilitated in improving the vegetative growth of plants particularly height of the plants. Jiang and Hull (1998) reviewed that crop yields were often correlated with high NR- activity assayed in the leaves of barley,
cotton, maize, pearl millet, sorghum and wheat. Kiran Kumar (2007) obtained significant results on improvement of growth, growth components and fruit yield in tomato plants treated with PGPR strains at all intervals of time and pointed out that this may be due to high production of phytohormone by the PGPR strains. Earnapalli (2005) also observed similar stimulated growth and yield of tomato after treatment with PGPR strains. Muis and Quimio (2006) observed the suppression of banded leaf sheath disease caused by *Rhizoctonia solani* in maize plants treated with formulated *Bacillus subtilis* BR23 which resulted in the grain yield increase by 27% compared to control. Farouk et al. (2008) reported that cucumber plants treated with elicitors such as SA and chitosan enhances yield increase. Present study demonstrates that the elicitor treated cumin plants performed better due to higher level of NR, NiR, and total carbohydrate and resulted in more yield than untreated control plants. This strongly supports the results obtained in the initial experiments at the laboratory level and experimental plots. Moreover, positive results obtained in the 1<sup>st</sup> field study supports the conclusion of the work done in this study that fungal elicitors provoke the onset of SAR in cumin plants.

Establishment of SAR in cumin plants results in the more yield in terms of quantity and quality. This definitely helps the farmer in fetching the better market price. This also helps in meeting the demand for local consumption in the country as well as to satisfy the export requirements.

**Essential oil extraction and identification of cuminaldehyde concentration**

Essential oil of cumin has great efficiency in controlling *Fusarium* spp. and inducing resistance toward pathogens (Agrawal, 1996; Hashem et al., 2010). Antimicrobial testing performed by Jirovetz et al., (2005) showed high activity of the essential oil of cumin against the mold *Aspergillus niger*, the Gram (+) bacteria *Bacillus subtilis* and *Staphylococcus epidermidis* as well as the yeast *Saccharomyces cerevisiae* and *Candida albicans*. 4% cumin oil concentration resulted in growth reduction of *Fusarium oxysporum*, *F. moniliforme*, *F. equiseti*, *F. solani*, *F. dimerum* and *F. equiseti* (Hashem et al., 2010). Marjanalo et al., (2009) reported that storage life of the strawberry fruits was increased by the use of cumin essential oil significantly by inhibition of fungal infection. Karbin et al., (2009) showed the potential antifungal activity of the essential oils from *Cuminum cyminum* which
terminates conidial germination and germ tube elongation when different concentrations of the essential oil were tested against *Aspergillus flavus* in-vitro. *C. cyminum* essential oil exhibited higher antibacterial and antifungal activities with a high effectiveness against *Vibrio* spp. strain (Hajlaoui et al., 2010). Moreover, essential oil of cumin also gives potential resistance to plants against *F. oxysporum f. sp. cumini*. Looking into the antimicrobial properties of cumin essential oil, it was extracted and concentration was determined among the treated and untreated plants of cumin to ascertain the quality in the present study.

In the 2nd field experiment conducted at the Sarla village of the Ahmedabad district during 2012-13 generated positive results in regards to cumin essential oil. Essential oil of cumin holds immense antibacterial, fungicidal and pesticidal property against wide array of pathogenic microbes. Marked difference in the quantity of extracted essential oil from harvested cumin seeds from the treated and untreated plants was seen in the present study (Table 33). Seeds collected at the time of harvesting from FCFs treated plants resulted in highest production of cumin oil compared to headline treated and control plants as shown below in table 33.

Table 33: Percentage yield of essential oil extracted from cumin seeds from treated and untreated plants (2012-13).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatments</th>
<th>Quantity (ml)/ gm of sample</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.20</td>
<td>4.02 %</td>
</tr>
<tr>
<td>2</td>
<td>Fungal elicitors</td>
<td>0.37</td>
<td>5.72 %</td>
</tr>
<tr>
<td>3</td>
<td>Headline</td>
<td>0.27</td>
<td>4.54 %</td>
</tr>
</tbody>
</table>

In the 3rd field experiment conducted at the Sarla village of Ahmedabad district, noticeable difference in the quantity of extracted essential oil from harvested cumin seeds was seen in the present study as shown in table 34. Seeds collected from 10% FOFCF treated plants resulted in highest production of cumin oil compared to control and other treated plants. 5% ABFCF has also showed better response in % yield of essential oil.
Table 34: Percentage yield of essential oil extracted from cumin seeds harvested from treated and untreated plants (2013-14).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatments</th>
<th>Quantity (ml)/ gm of sample</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.117</td>
<td>3.85 %</td>
</tr>
<tr>
<td>2</td>
<td>0.1% Headline</td>
<td>0.168</td>
<td>4.58 %</td>
</tr>
<tr>
<td>3</td>
<td>0.1% Headline (FO)</td>
<td>0.132</td>
<td>4.09 %</td>
</tr>
<tr>
<td>4</td>
<td>0.1% Headline (AB)</td>
<td>0.137</td>
<td>4.12 %</td>
</tr>
<tr>
<td>5</td>
<td>FOFCF</td>
<td><strong>0.222</strong></td>
<td>6.92 %</td>
</tr>
<tr>
<td>6</td>
<td>FO (AB) FCF</td>
<td>0.175</td>
<td>4.62 %</td>
</tr>
<tr>
<td>7</td>
<td>ABFCF</td>
<td>0.190</td>
<td>6.44 %</td>
</tr>
<tr>
<td>8</td>
<td>AB (FO) FCF</td>
<td>0.180</td>
<td>6.35 %</td>
</tr>
</tbody>
</table>

The essential oil quantity has increased in treated plants in comparison with control plants. Moreover, according to the Indian standards of seed spices, aroma of the essential oil is important. The aroma is dependent upon essential oil’s important constituent cuminaldehyde. The standards also suggest that the special seed variety, essential oil should be minimum 1.5%/ml/100 gm. In the results of field experiment 2 and 3, essential oil production was recorded 3.7% and 2.2% respectively (Tables 33 & 34) in the seeds harvested from the plants treated with FOFCF which is almost double than the essential oil recovered from the respective control seeds. Moreover, ABFCF treated plants in case of 3rd field experiment have also generated adequate amount of essential oil compared to control and headline treated seeds. Increased oil content which is one of the important quality measure for the essential volatile oil, remunerably helps the farmer to procure the price benefits. Thus, it can be concluded that fungal elicitor also helps in developing the quality along with the quantity of the essential oil.

The elicitor treated plants have shown highest induction in defense related enzymes, marked increment in physiological parameters and highest percentage yield of cumin essential oil. The results obtained in the present study clearly indicate the
role of elicitors obtained from *Fusarium oxysporum f. sp. cumini* and *Alternaria burnsii* in growth and development of the plants. Increased resistance of cumin plants also helps plants in sustaining array of pathogen and unfavourable climatic condition which leads to less mortality and more yield.

**Determination of cuminaldehyde concentration:**

After the GC analysis of extracted cumin essential oils from treated and untreated seeds harvested from the 2\textsuperscript{nd} field experiment conducted at Sarla village of Ahmedabad district during 2012-13, very interesting results were obtained. Upon comparing the retention times of samples with that of standard, peak of cuminaldehyde was identified. Peak of elicitor treated sample was higher than that of headline treated and untreated samples which clearly indicates the elicitor’s effect on increase in cuminaldehyde concentration. Moreover, area covered by elicitor treated samples was also more than the essential oils extracted from headline treated and control seeds. The retention time, area and calculated concentration is given in the Table 35.

Table 35: Gas chromatographic analysis for cuminaldehyde concentration in seeds collected from treated and untreated plants (2012-13).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>Retention Time (Min)</th>
<th>Area</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard</td>
<td>6.351</td>
<td>4807610.18</td>
<td>99.99</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>6.396</td>
<td>428.07</td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td>Fungal elicitors</td>
<td>6.407</td>
<td><strong>15075.18</strong></td>
<td><strong>31.3</strong></td>
</tr>
<tr>
<td>4</td>
<td>Headline</td>
<td>6.415</td>
<td>5434.89</td>
<td>11.3</td>
</tr>
</tbody>
</table>
Chromatograph showing appearance of cuminaldehyde in untreated control plant’s seed sample (2012-13):

Chromatograph showing appearance of cuminaldehyde in FCFs treated plant’s seed sample (2012-13):
Chromatograph showing appearance of cuminaldehyde in Headline treated plant’s seed sample (2012-13):

Cuminaldehyde concentration was measured from the samples retrieved after conducting the 3rd field experiment at Sarla village, Ahmedabad district in the year 2013-14. GC analysis data shown in table 36 are of treated and untreated samples on retention time, area and calculated concentration of cuminaldehyde. Seeds harvested from FOFCF treated plants showed highest production of cuminaldehyde compared to other treatments. Overall, all the fungal elicitor treated plants showed moderate increase in cuminaldehyde production compared to control plants. Increase in cuminaldehyde concentration naturally provide defense against the pathogens. Its induced level in the present study after the application of fungal elicitors confirms their positive impact over cumin plants.
Table 36: Gas chromatographic analysis for cuminaldehyde concentration in seeds harvested from treated and untreated plants (2013-14).

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>Retention Time (Min)</th>
<th>Area</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard</td>
<td>12.01</td>
<td>8079612.60</td>
<td>99.99</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>12.11</td>
<td>1361071.05</td>
<td>16.84</td>
</tr>
<tr>
<td>3</td>
<td>0.1% Headline</td>
<td>12.09</td>
<td>1527998.41</td>
<td>18.91</td>
</tr>
<tr>
<td>4</td>
<td>0.1% Headline (FO)</td>
<td>12.12</td>
<td>1604822.57</td>
<td>19.86</td>
</tr>
<tr>
<td>5</td>
<td>0.1% Headline (AB)</td>
<td>12.06</td>
<td>1432311.38</td>
<td>17.72</td>
</tr>
<tr>
<td>6</td>
<td><strong>FOFCF</strong></td>
<td>12.05</td>
<td><strong>2303984.96</strong></td>
<td><strong>28.51</strong></td>
</tr>
<tr>
<td>7</td>
<td>FO (AB) FCF</td>
<td>12.06</td>
<td>1586825.98</td>
<td>19.63</td>
</tr>
<tr>
<td>8</td>
<td>ABFCF</td>
<td>12.09</td>
<td>2215329.20</td>
<td>27.41</td>
</tr>
<tr>
<td>9</td>
<td>AB (FO) FCF</td>
<td>12.13</td>
<td>1625037.90</td>
<td>20.11</td>
</tr>
</tbody>
</table>

Chromatograph showing appearance of cuminaldehyde in untreated control plant’s seed sample (2013-14):
Chromatograph showing appearance of cuminaldehyde in 0.1% Headline treated plant’s seed sample (2013-14):

Chromatograph showing appearance of cuminaldehyde in 0.1 % Headline (FO) treated plant’s seed sample (2013-14):

Chromatograph showing appearance of cuminaldehyde in 0.1% Headline (AB) treated plant’s seed sample (2013-14):
Results and Discussion

Chromatograph showing appearance of cuminaldehyde in FOFCF treated plant’s seed sample (2013-14):

Chromatograph showing appearance of cuminaldehyde in FO (AB) FCF treated plant’s seed sample (2013-14):

Chromatograph showing appearance of cuminaldehyde in ABFCF treated plant’s seed sample (2013-14):
Results and Discussion

Chromatograph showing appearance of cuminaldehyde in AB (FO) FCF treated plant’s seed sample (2013-14):

The results obtained in the present study confirm the positive impact of SAR in disease resistance. Induction in various defense related enzymes, PR proteins and phenols as well as cuminaldehyde provide ample immunity to the plants to deal with the pathogens. Because of this, better growth and development as well as higher yield can be achieved which was seen in the present study. The aroma of the cumin essential oil is highly dependent upon its important constituent cuminaldehyde. The results obtained in the present study noticeably prove the increase in the cuminaldehyde concentration. More cuminaldehyde will provide strong aroma which is very much essential according to the marketing point of view, especially for exporting the commodity. Seeds obtained from the plants after fungal elicitor treatment meets the quality requirements for the export by the exporters.

Comparative study of control, infected, nutrient deficient and elicitor treated plants:

The resistance in plants is a result of host-pathogen interaction which involve morphological and biochemical changes (Singh et al., 2011). Comparison of defense related enzymes and total protein, total phenol, reducing sugar and non reducing sugar concentrations were performed in \textit{Fusarium oxysporum} as well as \textit{Alternaria burnsidei} infected plants, nutrient deficient plants, control and 0.1% headline, 1.0 % monitor and 10% FCF of \textit{F. oxysporum} treated plants. From the study, it was found that the pathogen infected plants (Fig. 31D-E) showed less enzyme activity than the control
and treated plants. Nutrient deficient plants also showed reduction in enzyme activity compared to control and treated plants (Figs. 96A-D, 97A-D, 98A-C).

It can be interpreted from the above results that due to disease condition, there was malfunctioning of metabolic activities which resulted in decrease activity of defense related enzymes in infected and nutrient deficient plants. Similar findings were observed by several researchers where disease incidence has played a role in death of the plants by inactivating the phenyl propanoid pathway. Chatterjee and Ghosh (2008) found that mesta plant infected with yellow vein mosaic virus showed decrease in the phenol level compared control. Khan et al. (2001) also noted that on infection of *Drechslera sorghicola* in sorghum lead to insufficient oxidation of phenols which was unable to control the pathogen spread. Report showed that the decrease in the POX activity in Mesta and Sorghum plant infected with yellow vein mosaic virus and *Drechslera sorghicola* respectively, which has key role in lignin and protein synthesis and also regulate the phenyl propanoid pathway. Chatterjee and Ghosh (2008) have also reported that the decrease in level of protein upon infection due to its denaturation cause early death of the plants. The decrease in level of total sugar particularly non-reducing sugar was seen in acid lime by *Xanthomonas axonopodis pv. citri* (XAC) and in mustard plant infected by *Albugo candida* (Manonmani et al., 2009; Singh et al., 2011). In this study also reduced enzyme activities were observed in *Fusarium* and *Alternaria* infected plants to treated plants with elicitors. The enhanced enzyme activities in elicitor treated plants than control plants indicate that the plants are synthesizing the key molecules necessary to develop systemic resistance (SAR) against pathogens.

**Bioassay of biocontrol agents and phytoalexins extracted from treated plant samples:**

Phytoalexins are low molecular weight antimicrobial compounds accumulated in plants after exposure to microorganisms. They are found in a wide variety of plants. These phytoalexins have been thought to play a significant role in the defense strategies of plants. The significance of phytoalexins should be sought not only in their antifungal activities or in the timing of accumulation. Theoretically some metabolic intermediates may enter and regulate microbial cells to become nonaggressive without killing them and without accumulating in detectable amounts.
at a certain stage of infection. The accumulation may be a consequence of amplified synthesis induced by a nonaggressive pathogen (Ouchi, 1983). The experiments performed in the present study clearly confirm the induction of SAR in cumin plants. Phytoalexins are produced in high amount as the onset of SAR follows the pathway that produces several antimicrobial compounds. To check their efficacy in controlling the pathogenic growth, this experiment was performed.

Table 37: Zone of inhibition against *F. oxysporum* and *A. burnsii* by different biocontrol agents and phytoalexins of treated plant extracts

<table>
<thead>
<tr>
<th>Test Pathogens</th>
<th>Treatments</th>
<th>Zone of inhibition</th>
<th>Phytoalexin extracted from leaf after treatment with various elicitors</th>
<th>Zone of inhibition against</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>F. oxysporum</em></td>
</tr>
<tr>
<td><em>F. oxysporum f. sp. cumini</em></td>
<td>Control (DMSO)</td>
<td>0.0mm</td>
<td>Control (D. Water)</td>
<td>0.0mm</td>
</tr>
<tr>
<td></td>
<td><strong>Headline (100 mg/ml)</strong></td>
<td><strong>22.0mm</strong></td>
<td>0.1% Headline</td>
<td>10.0mm</td>
</tr>
<tr>
<td></td>
<td>Monitor (100 mg/ml)</td>
<td>3.0mm</td>
<td>1.0% monitor</td>
<td>8.00mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>10% FOFCF</strong></td>
<td><strong>17.0mm</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>5% ABFCF</strong></td>
<td>12.0mm</td>
</tr>
<tr>
<td><em>Alternaria burnsii</em></td>
<td>Control (DMSO)</td>
<td>0.0mm</td>
<td>Control (D. water)</td>
<td>0.0mm</td>
</tr>
<tr>
<td></td>
<td><strong>Headline (100 mg/ml)</strong></td>
<td><strong>12.0mm</strong></td>
<td>0.1% Headline</td>
<td>4.0mm</td>
</tr>
<tr>
<td></td>
<td>Monitor (100 mg/ml)</td>
<td>5.0mm</td>
<td>1.0% monitor</td>
<td>5.0mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><strong>10% FOFCF</strong></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
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<td><strong>5% ABFCF</strong></td>
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Antimicrobial activity of selected biocontrol agents viz. headline and monitor was performed against *F. oxysporum f. sp. cumini* and *A. burnsii*. Headline resulted in highest zone of inhibition than monitor against both organisms, while no zone of
inhibition was observed in DMSO used as control. 22 mm of zone of inhibition was observed against *F. oxysporum f. sp. cumini* (Fig. 99A) and 12 mm zone of inhibition against *Alternaria burnsii* by headline (Fig. 99B) (Table 37). Bioassay performed with plant extracts prepared from leaves treated with different FCFs and biocontrol agents also showed varied zone of inhibition (Table 37). 10% FOFCF and 5% ABFCF treated plant leaf extracts showed 17 mm and 12 mm zone of inhibition respectively (Fig. 99C, 99E) against *F. oxysporum f. sp. cumini* while 7.0 mm zone of inhibition against *A. burnsii* (Fig. 99D, 99F) in both FCF treatments (Table 37).

Results obtained in this assay concludes that production of various phytoalexins may have induced after the treatment with selected elicitors and especially FCFs. Induction in the phytoalexins amount can easily be correlated with the induction of SAR. Onset of SAR naturally provides strongest line of defense to the cumin plants to combat devastating fungal (wilt and blight) diseases.
Figure 20: Selected cumin seeds variety, morphological characters of pathogenic fungal strains: *Fusarium oxysporum f. sp. cumini* and *A. burnsii*

A: Cumin seeds of GC-2 variety

B: Cumin seeds of GC-4 variety

C: *F. oxysporum f. sp. cumini* growing on potato dextrose agar medium

D: *F. oxysporum f. sp. cumini* growing in potato dextrose broth

E: Sickle shaped macro and microconidia of *F. oxysporum f. sp. cumini* stained with lactophenol cotton blue

F: *Alternaria burnsii* growing on potato dextrose agar medium

G: *A. burnsii* fungal culture filtrate (FCF) collected after 23rd day of growth

H: Septate conidia of *A. burnsii* stained with lactophenol cotton blue
Figure 21: Selected microorganisms, their morphological characteristics and biocontrol agents

A: Bacillus subtilis growing on nutrient agar medium

B: B. subtilis growing in nutrient broth medium

C: Rod shaped Bacilli observed after Gram’s staining

D: Trichoderma harzianum growing on potato dextrose agar medium

E: T. harzianum growing on potato dextrose broth

F: Chlamydospores of T. harzianum observed in microscope after staining with lactophenol cotton blue

G: Selected biocontrol agent: Headline

H: Selected biocontrol agent: Monitor
Figure 22: Estimation of protein concentration in FCFs and CCF of selected microorganisms prepared for the study at different time interval

A: *F. oxysporum f. sp. cumini* FCF
B: *A. burnii* FCF
C: *T. harzianum* FCF
D: *B. subtilis* CCF
Figure 22
Figure 23: (A-C): Pathogenicity test of *F. oxysporum f. sp. cumini* and *A. burnsii* on healthy cumin plant leaves and (D-E): SDS-PAGE analysis of crude, dialyzed and column purified elicitor protein of selected fungi.

A: Cumin leaves incubated with D/W for pathogenicity test

B & C: Appearance of symptoms on cumin leaves (yellowing & browning) when incubated with *F. oxysporum f. sp. cumini* and *A. burnsii* fungal spores for pathogenicity test

D: Glycoprotein staining with periodic acid-Schiff’s reagent of *F. oxysporum f. sp. cumini* FCF sample on SDS-PAGE

E: Glycoprotein staining with periodic acid-Schiff’s reagent of *A. burnsii* FCF sample on SDS-PAGE

M- Marker, C- Crude FCF, D- Dialyzed FCF, CP- Column purified 7th fraction
Figure 24: Standardization of concentration (A-B) and treatment time (C-D) by using FCFs of *F. oxysporum f. sp. cumini*, *T. harzianum* and *B. subtilis* CCF as elicitors.

A: Estimation of PAL activity in seeds treated with different concentrations of elicitors.

B: Estimation of POX activity in seeds treated with different concentrations of elicitors.

C: Estimation of PAL activity in seedlings treated with 10% *F. oxysporum f. sp. cumini* FCF, 5% *B. subtilis* CCF and 20% *T. harzianum* FCF at different time intervals.

D: Estimation of POX activity in seeds treated with 10% *F. oxysporum f. sp. cumini* FCF, 5% *B. subtilis* CCF and 20% *T. harzianum* FCF at different time intervals.
Figure 24
Figure 25: Standardization of (A-B) concentration of FCFs of *A. burnsii* (ABFCF) and treatment time (C-D) with FOFCF, ABFCF and MIX FCF.

A: Estimation of PAL activity in seedlings treated with different concentrations of ABFCF.

B: Estimation of POX activity in seedlings treated with different concentrations of ABFCF.

C: Estimation of PAL activity of seeds treated with 10% *F. oxysporum* f. sp. *cumini* FCF, 5% *A. burnsii* FCF and MIX FCF for different time intervals.

D: Estimation of POX activity of seeds treated with 10% *F. oxysporum* f. sp. *cumini* FCF, 5% *A. burnsii* FCF and MIX FCF for different time intervals.
Figure 25
Figure 26: Standardization of seed treatment time with various elicitors by estimating the PAL, POX enzyme activities.

A: PAL activity in seeds treated with different concentrations of 10% FCF of *F. oxysporum f. sp. cumini* and 5% FCF of *A. burnsii.*

B: POX activity in seeds treated with different concentrations of 10% FCF of *F. oxysporum f. sp. cumini* and 5% FCF of *A. burnsii.*

C: PAL activity in seedlings treated with 10% FCF, 0.1% & 1.0% Headline and 0.5% & 1.0% concentration of Monitor at different time intervals.

D: POX activity in seeds treated with 10% FCF, 0.1% & 1.0% Headline and 0.5% & 1.0% concentration of Monitor at different time intervals.
Figure 27: Seed germination after treatment with 10% FOFCF, 5% ABFCF and MIX FCF at lab condition.

A: Radical emergence in GC-2 variety seeds after 3 days of treatment with various elicitors

B: Radical emergence in GC-4 variety seeds after 3 days of treatment with various elicitors

C: Radical emergence in GC-2 variety seeds after 5 days of treatment with various elicitors

D: Radical emergence in GC-4 variety seeds after 5 days of treatment with various elicitors

E: Radical emergence in GC-2 variety seeds after 7 days of treatment with various elicitors

F: Radical emergence in GC-4 variety seeds after 7 days of treatment with various elicitors
Figure 28: Seed germination assay performed in pots containing garden soil after treatment with 10% FOFCF, 5% ABFCF and MIX FCF.

A: 21 days old seedlings of GC-2 variety growing in garden soil

B: 21 days old seedlings of GC-4 variety growing in garden soil
Figure 29: Cumin plants of GC-2 and GC-4 variety growing in *F. oxysporum f. sp. cumini* infested soil after treatment with different elicitors.

A: Seeds of GC-2 variety treated with 10% FOFCF and germinated in FO infested soil after 21 days.

B: Seeds of GC-4 variety treated with 10% FOFCF and germinated in FO infested soil after 21 days.

C: Seeds of GC-2 variety treated with 5% *B. subtilis* CCF and germinated in FO infested soil after 21 days.

D: Seeds of GC-4 variety treated with 5% *B. subtilis* CCF and germinated in FO infested soil after 21 days.

E: Seeds of GC-2 variety treated with 20% *T harzianum* and germinated in FO infested soil after 21 days.

F: Seeds of GC-4 variety treated with 20% *T harzianum* FCF and germinated in FO infested soil after 21 days.
Figure 30: Cumin seeds treated with elicitors (10% FOFCF, 5% ABFCF and MIX FCF) and grown in fungal infested soil.

A: 21 days old GC-2 variety plants treated with elicitors growing in pots containing soil infested with *F. oxysporum f. sp. cumini*

B: 21 days old GC-4 variety plants treated with elicitors growing in pots containing soil infested with *F. oxysporum f. sp. cumini*

C: 21 days old GC-2 variety plants treated with elicitors growing in pots containing soil infested with *A. burnsii*

D: 21 days old GC-4 variety plants treated with elicitors growing in pots containing soil infested with *A. burnsii*
Figure 31: View of cumin plants growing in experimental plots and farmer’s field during various conducted experiments.

A: Cumin plants in the experimental plot sown after seed treatment with elicitors and biocontrol agents.

B: Application of elicitors and biocontrol agents through foliar spray on cumin plants growing in the experimental plot.

C: Healthy cumin plants growing in experimental plot.

D: Control plants treated with D/W growing in the field.

E: Healthy cumin plants after fungal elicitor treatment growing in the field.

F: *F. oxysporum f. sp. cumini* infected control plants growing in field.

G: *A. burnsii* infected control plants in field.
Figure 32: Estimation of defense related enzymes in GC-2 plants growing in infested soil after the treatment with FOFCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of PPO activity
Figure 32
Figure 33: Estimation of defense related enzymes, proteins and phenols in GC-2 plants growing in infested soil after the treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of β-1,3 glucanase activity

B: Change in Catalase activity

C: Induction of total protein concentration

D: Induction of total phenols concentration
Figure 33
Figure 34: Estimation of defense related enzymes in GC-4 plants growing in infested soil after the treatment with FOFCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction in PAL activity

B: Induction in POX activity

C: Induction in PPO activity
Figure 35: Estimation of defense related enzymes, proteins and phenols in GC-4 plants growing in infested soil after the treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of β-1,3 glucanase activity

B: Change in Catalase activity

C: Induction of total protein concentration

D: Induction of total phenols concentration
Figure 35
Figure 36: SDS-PAGE protein profiling of samples obtained from plant growing in infested soil after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF.

A: GC-2 plants

B: GC-4 plants

Lane 1- Marker; Lane 2- *F. oxysporum f. sp. cumini*, Lane 3- Control, Lane 4- *B. subtilis*, Lane 6- *T. harzianum*, Lane 7- Control

Arrows: indicate expression of protein bands
Figure 37: Estimation of defense related enzymes from leaf samples collected from GC-2 variety plants growing in soil infested with *F. oxysporum f. sp. cumini* after seed treatment with elicitors at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3 glucanase activity

D: Induction of PPO activity
Figure 37
Figure 38: Estimation of defense related enzymes and total protein concentration from leaf samples of GC-2 variety plants growing in soil infested with *F. oxysporum f. sp. cumini* after seed treatment with elicitors at different time interval.

A: Induction of Catalase activity

B: Induction of Nitrate reductase activity

C: Induction of Nitrite reductase activity

D: Induction of Total protein concentration
Figure 38
Figure 39: Estimation of total phenol, reducing and non reducing sugar content from leaf samples of GC-2 variety plants growing in soil infested with *F. oxysporum f. sp. cumini* after seed treatment with elicitors at different time interval.

A: Induction of Total phenol content

B: Induction of Reducing sugar content

C: Induction of Non reducing sugar content
Figure 39
Figure 40: Estimation of defense related enzymes from leaf samples of GC-4 variety plants growing in soil infested with *F. oxysporum f. sp. cumini* after seed treatment with elicitors at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3 glucanase activity

D: Induction of PPO activity
Figure 40
Figure 41: Estimation of defense related enzymes and total protein concentration from leaf samples of GC-4 variety plants growing in soil infested with *F. oxysporum f. sp. cumini* after seed treatment with elicitors at different time interval.

A: Induction of Catalase activity

B: Induction of Nitrate reductase activity

C: Induction of Nitrite reductase activity

D: Induction of Total protein concentration
Figure 41
Figure 42: Estimation of total phenol, reducing and non reducing sugar content from leaf samples of GC-4 variety plants growing in soil infested with *F. oxysporum f. sp. cumini* after seed treatment with elicitors at different time interval.

A: Induction of Total phenol content

B: Induction of Reducing sugar content

C: Induction of Non reducing sugar content
Figure 42
Figure 43: Estimation of defense related enzymes from leaf samples of GC-2 variety plants growing in soil infested with *A. burnsii* after seed treatment with elicitors at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3 glucanase activity

D: Induction of PPO activity
Figure 43
Figure 44: Estimation of defense related enzymes and total protein concentration from leaf samples of GC-2 variety plants growing in soil infested with *A. burnsii* after seed treatment with elicitors at different time interval.

A: Induction of Catalase activity

B: Induction of Nitrate reductase activity

C: Induction of Nitrite reductase activity

D: Induction of Total protein concentration
Figure 44
Figure 45: Estimation of total phenol, reducing and non reducing sugar content from leaf samples of GC-2 variety plants growing in soil infested with *A. burnsii* after seed treatment with elicitors at different time interval.

A: Induction of Total phenol content

B: Induction of Reducing sugar content

C: Induction of Non reducing sugar content
Figure 45
Figure 46: Estimation of defense related enzymes from leaf samples of GC-4 variety plants growing in soil infested with *A. burnsii* after seed treatment with elicitors at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3 glucanase activity

D: Induction of PPO activity
Figure 46
Figure 47: Estimation of defense related enzymes and total protein concentration from leaf samples of GC-4 variety plants growing in soil infested with *A. burnsii* after seed treatment with elicitors at different time interval.

A: Induction of Catalase activity

B: Induction of Nitrate reductase activity

C: Induction of Nitrite reductase activity

D: Induction of Total protein concentration
Figure 47
Figure 48: Estimation of total phenol, reducing and non reducing sugar content from leaf samples of GC-4 variety plants growing in soil infested with *A. burnsii* after seed treatment with elicitors at different time interval.

A: Induction of Total phenol content

B: Induction of Reducing sugar content

C: Induction of Non reducing sugar content
Figure 48
Figure 49: Protein profiling by SDS-PAGE of the leaf samples collected from the plants growing in soil infested with *F. oxysporum f. sp. cuminii* after seed treatment with elicitors at different time interval.

A: Protein profiling of GC-2 variety

B: Protein profiling of GC-4 variety

Lane 1- Marker, Lane 2- Control, Lane 3- 10% *F. oxysporum* FCF, Lane 4- MIX FCF, Lane 5- Control, Lane 6- 5% ABFCF
Figure 50: Protein profiling by SDS-PAGE of the leaf samples collected from the plants growing in soil infested with *A. burnsii* after seed treatment with elicitors at different time interval.

A: Protein profiling of GC-2 variety

B: Protein profiling of GC-4 variety

Lane 1- Marker, Lane 2- Control, Lane 3- 10% *F. oxysporum* FCF, Lane 4- MIX FCF, Lane 5- Control, Lane 6- 5% ABFCF
Figure 51: Estimation of defense related enzymes in GC-2 plants growing in experimental plot after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of PPO activity
Figure 52: Estimation of enzymes, proteins and phenols in GC-2 plants growing in experimental plot after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of β-1,3 glucanase activity

B: Change in Catalase activity

C: Induction of total protein concentration

D: Induction of total phenols concentration
Figure 52
Figure 53: Estimation of defense related enzymes in GC-4 plants growing in experimental plot after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of PPO activity
Figure 53
Figure 54: Estimation of enzymes, proteins and phenols in GC-4 plants growing in experimental plot after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of β-1,3 glucanase activity

B: Change in Catalase activity

C: Induction of total protein concentration

D: Induction of total phenols concentration
Figure 54
Figure 55: SDS-PAGE analysis of protein profiling of samples obtained from plants growing in experimental plot after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF.

A: GC-2 plants

B: GC-4 plants

Lane 1- Marker; Lane 2- *F. oxysporum f. sp. cumini*, Lane 3- Control, Lane 4- *B. subtilis*, Lane 6- *T. harzianum*, Lane 5, 7- Control

Arrows: indicate expression of protein bands
Figure 56: Estimation of defense related enzymes in GC-2 plants growing in experimental plot after foliar spray treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of PPO activity
Figure 57: Estimation of enzymes, proteins and phenols in GC-2 plants growing in experimental plot after foliar spray treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of β-1,3 glucanase activity

B: Change in Catalase activity

C: Induction of total protein concentration

D: Induction of total phenols concentration
Figure 58: Estimation of defense related enzymes in GC-4 plants growing in experimental plot after foliar spray treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of PPO activity
Figure 58
Figure 59: Estimation of enzymes, proteins and phenols in GC-4 plants growing in experimental plot after foliar spray treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF at different time interval.

A: Induction of β-1,3 glucanase activity

B: Change in Catalase activity

C: Induction of total protein concentration

D: Induction of total phenols concentration
Figure 59
Figure 60: SDS-PAGE analysis of protein profiling of samples collected from plants growing in experimental plot after foliar spray treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF.

A: GC-2 plants

B: GC-4 plants

Lane 1- Marker; Lane 2- *F. oxysporum f. sp. cumini*, Lane 3- Control, Lane 4- *B. subtilis*, Lane 5- control, Lane 6- *T. harzianum*, Lane 7- Control

Arrows: indicate expression of protein bands
Figure 61: Estimation of defense related enzymes in GC-2 variety of cumin plants growing in second experimental plot experiment after foliar spray treatment with 10% FOFCF, 0.1% and 1.0% headline, 0.5% and 1.0% monitor at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3-glucanase activity

D: Induction of PPO activity
Figure 62: Estimation of defense related enzymes and total protein concentration in GC-2 variety of cumin plants growing in second experimental plot experiment after foliar spray treatment with 10%FOFCF, 0.1% and 1.0% headline, 0.5% and 1.0% monitor at different time interval.

A: Induction of catalase activity

B: Induction of nitrate reductase activity

C: Induction of nitrite reductase activity

D: Induction of total protein concentration
Figure 62
Figure 63: Estimation of total phenol, reducing sugar and non-reducing sugar concentration in GC-2 variety of cumin plants growing in second experimental plot experiment after foliar spray treatment with 10% FOFCF, 0.1% and 1.0% headline, 0.5% and 1.0% monitor at different time interval.

A: Induction of total phenol concentration

B: Induction of reducing sugar concentration

C: Induction of non-reducing sugar concentration
Figure 63
Figure 64: Estimation of defense related enzymes in GC-4 variety of cumin plants growing in second experimental plot experiment after foliar spray treatment with 10%FOFCF, 0.1% and 1.0% headline, 0.5% and 1.0% monitor at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3-glucanase activity

D: Induction of PPO activity
Figure 64
Figure 65: Estimation of defense related enzymes and total protein concentration in GC-4 variety of cumin plants growing in second experimental plot experiment after foliar spray treatment with 10%FOFCF, 0.1% and 1.0% headline, 0.5% and 1.0% monitor at different time interval.

A: Induction of catalase activity

B: Induction of nitrate reductase activity

C: Induction of nitrite reductase activity

D: Induction of total protein concentration
Figure 65
Figure 66: Estimation of total phenol, reducing sugar and non-reducing sugar concentration in GC-4 variety of cumin plants growing in second experimental plot experiment after foliar spray treatment with 10%FOFCF, 0.1% and 1.0% headline, 0.5% and 1.0% monitor at different time interval.

A: Induction of total phenol concentration

B: Induction of reducing sugar concentration

C: Induction of non-reducing sugar concentration
Figure 66
Figure 67: SDS-PAGE analysis of proteins and isozymes study from the plant samples treated with 0.1% and 1.0% headline, 0.5% and 1.0% monitor and 10% FOFCF in second experimental plot experiment.

A: Protein profiling of GC-2 variety treated plants

B: Protein profiling of GC-4 variety treated plants

Lane 1- Marker, Lane 2- Control, Lane 3- 0.1% Headline, Lane 4- 1.0% Headline, Lane 5- 0.5% Monitor, Lane 6- 1.0% Monitor, Lane 7- 10% FOFCF

C: Peroxidase profiling of GC-2 variety

D: Peroxidase profiling of GC-4 variety

Lane 1- Control, Lane 2- 0.1% Headline, Lane 3- 1.0% Headline, Lane 4- 0.5% Monitor, Lane 5- 1.0% Monitor, Lane 6- 10% FOFCF
Figure 67
Figure 68: Estimation of defense related enzymes in GC-2 variety cumin plants growing in third experimental plot experiment after two foliar spray treatment with FOFCF, ABFCF and MIX FCF at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3 glucanase activity

D: Induction of PPO activity
Figure 69: Estimation of defense related enzymes and total protein concentration in GC-2 variety cumin plants growing in third experimental plot experiment after two foliar spray treatment with FOFCF, ABFCF and MIX FCF at different time interval.

A: Induction of Catalase activity

B: Induction of Nitrate reductase activity

C: Induction of Nitrite reductase activity

D: Induction of Total protein concentration
Figure 69
Figure 70: Estimation of total phenol, reducing and non reducing sugar content in GC-2 variety cumin plants growing in third experimental plot experiment after two foliar spray treatment with FOFCF, ABFCF and MIX FCF at different time interval.

A: Induction of Total phenol content

B: Induction of Reducing sugar content

C: Induction of Non reducing sugar content
Figure 70
Figure 71: Estimation of defense related enzymes in GC-4 variety cumin plants growing in third experimental plot experiment after two foliar spray treatment with FOFCF, ABFCF and MIX FCF at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3 glucanase activity

D: Induction of PPO activity
Figure 71
Figure 72: Estimation of defense related enzymes and total protein concentration in GC-4 variety cumin plants growing in third experimental plot experiment after two foliar spray treatment with FOFCF, ABFCF and MIX FCF at different time interval.

A: Induction of Catalase activity

B: Induction of Nitrate reductase activity

C: Induction of Nitrite reductase activity

D: Induction of Total protein concentration
Figure 72
Figure 73: Estimation of total phenol, reducing and non reducing sugar content in GC-4 variety cumin plants growing in third experimental plot experiment after two foliar spray treatment with FOFCF, ABFCF and MIX FCF at different time interval.

A: Induction of Total phenol content

B: Induction of Reducing sugar content

C: Induction of Non reducing sugar content
Figure 74: Protein profiling through SDS-PAGE of the leaf samples collected from plants of both varieties growing in third experimental plot experiment after foliar spray treatments with elicitors.

A: Protein profiling of GC-2 variety after 1\textsuperscript{st} spray
B: Protein profiling of GC-4 variety after 1\textsuperscript{st} spray
C: Protein profiling of GC-2 variety after 2\textsuperscript{nd} spray
D: Protein profiling of GC-4 variety after 2\textsuperscript{nd} spray

Lane 1- Marker, 2- 10\% FOFCF, 3- Control, 4- 5\% ABFCF, 5- Control, 6- MIX FCF, 7- Control
Figure 75: Phenol profiling of samples obtained from plants growing in infested soil after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF.

A: GC-2 plants

B: GC-4 plants

C-D: Phenol profiling of samples obtained from plants growing in experimental plot after seed treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF.

C: GC-2 plants

D: GC-4 plants

E-F: Phenol profiling of samples collected from plants growing in experimental plot after foliar spray treatment with *F. oxysporum f. sp. cumini* FCF, *B. subtilis* CCF and *T. harzianum* FCF.

E: GC-2 plants

F: GC-4 plants

Lane 1- Control, Lane 2- *F. oxysporum f. sp. cumini*, Lane 3- Control, Lane 4- *B. subtilis*, Lane 5- Control, Lane 6- *T. harzianum*
Figure 76: Phenol profiling of samples obtained from plants growing in infested soil after seed treatment with 10% FOFCF, 0.1% & 1.0% headline and 0.5% & 1.0% monitor.

A: GC-2 plants

B: GC-4 plants

Lane 1- Control, Lane 2- 0.1% headline, Lane 3- 1.0% headline, Lane 4- Control, Lane 5- 0.5% monitor, Lane 6- 1.0% monitor, Lane 7- Control, Lane 8- 10% FOFCF
Figure 77: Estimation of defense related enzymes in field grown 25 days old GC-2 variety cumin plants at Shantipura village after foliar spray treatment with 10% FOFCF, 0.1% and 1.0% headline at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3-glucanase activity

D: Induction of PPO activity
Figure 78: Estimation of defense related enzymes in field grown 25 days old GC-2 variety cumin plants at Shantipura village after foliar spray treatment with 10% FOFCF, 0.1% and 1.0% headline at different time interval.

A: Induction of catalase activity

B: Induction of nitrate reductase activity

C: Induction of nitrite reductase activity

D: Induction of total protein concentration
Figure 78
Figure 79: Estimation of defense related enzymes in field grown 25 days old GC-2 variety cumin plants at Shantipura village after foliar spray treatment with 10% FOFCF, 0.1% and 1.0% headline at different time interval.

A: Induction of total phenol concentration

B: Induction of reducing sugar concentration

C: Induction of non-reducing sugar concentration
Figure 80: Estimation of defense related enzymes in field grown 25 days old GC-4 variety cumin plants at Shantipura village after foliar spray treatment with 10% FOFCF, 0.1% and 1.0% headline at different time interval.

A: Induction of PAL activity

B: Induction of POX activity

C: Induction of β-1,3-glucanase activity

D: Induction of PPO activity
Figure 80
Figure 81: Estimation of defense related enzymes in field grown 25 days old GC-4 variety cumin plants at Shantipura village after foliar spray treatment with 10% FOFCF, 0.1% and 1.0% headline at different time interval.

A: Induction of catalase activity
B: Induction of nitrate reductase activity
C: Induction of nitrite reductase activity
D: Induction of total protein concentration
Figure 81
Figure 82: Estimation of defense related enzymes in field grown 25 days old GC-4 variety cumin plants at Shantipura village after foliar spray treatment with 10% FOFCF, 0.1% and 1.0% headline at different time interval.

A: Induction of total phenol concentration

B: Induction of reducing sugar concentration

C: Induction of non-reducing sugar concentration
Figure 82
Figure 83: Effect of elicitors on height and number of umbels in field grown cumin plants at Shantipura village.

A: Control and 10% FOFCF treated GC-2 plants
B: Control and 0.1% headline treated GC-2 plants
C: Control and 1.0% headline treated GC-2 plants
D: Control and 10% FOFCF treated GC-4 plants
E: Control and 0.1% headline treated GC-4 plants
F: Control and 1.0% headline treated GC-4 plants
Figure 84: Estimation of defense related enzymes in field grown GC-2 variety cumin plants at Sarla village after two foliar spray treatment with 10% FOFCF, 0.1% headline and control (DW) at different time interval.

A: Induction of phenylalanine ammonia lyase (PAL) activity

B: Induction of peroxidase (POX) activity

C: Induction of β-1,3 glucanase activity

D: Induction of polyphenol oxidase (PPO) activity
Figure 84
Figure 85: Estimation of defense related enzymes in field grown GC-2 variety cumin plants at Sarla village after two foliar spray treatment with 10% FOFCF, 0.1% headline and control (DW) at different time interval.

A: Induction of catalase activity

B: Induction of nitrate reductase (NRA) activity

C: Induction of nitrite reductase (NIR) activity
Figure 85
Figure 86: Estimation of defense related enzymes in field grown GC-2 variety cumin plants at Sarla village after two foliar spray treatment with 10% FOFCF, 0.1% headline and control (DW) at different time interval.

A: Induction of total protein concentration

B: Induction of total phenol concentration

C: Induction of reducing sugar concentration

D: Induction of non-reducing sugar concentration
Figure 86
Figure 87: A: Protein profiling of treated plants with different elicitors and B: effect of elicitors and 0.1% headline on physiological parameters of cumin plants grown in the farmer’s field at Sarla village.

A: SDS-PAGE profiling of induced PR proteins in FCF, headline treated and untreated plant samples after both treatments.

Lane 1- Marker, Lane 2- Control (1st spray), Lane 3- Headline (1st spray), Lane 4- Fusarium oxysporum f. sp. cuminii FCF (1st spray), Lane 5- Control (1st spray), Lane 6- Headline (2nd spray), Lane 7- Alternaria burnetii FCF (2nd spray)

Induced additional protein bands

B: Height and number of branches and umbels in control and FCF (FOFCF+ABFCF) treated GC-2 plants

C: Height and number of branches and umbels in control and 0.1% headline treated GC-2 plants
Figure 88: Estimation of defense related enzymes in field grown GC-2 variety cumin plants at Sarla village after 1st foliar spray treatment (25 DAS) with 10% FOFCF, 5% ABFCF, 0.1% headline and control (D/W) at different time interval.

A: Induction of phenylalanine ammonia lyase (PAL) activity

B: Induction of peroxidase (POX) activity

C: Induction of β-1,3 glucanase activity

D: Induction of polyphenol oxidase (PPO) activity
Figure 88
Figure 89: Estimation of defense related enzymes in field grown GC-2 variety cumin plants at Sarla village after 1st foliar spray treatment (25 DAS) with 10% FOFCF, 5% ABFCF, 0.1% headline and control (D/W) at different time interval.

A: Induction of catalase activity

B: Induction of nitrate reductase (NRA) activity

C: Induction of nitrite reductase (NIR) activity
Figure 89
Figure 90: Estimation of defense related enzymes in field grown GC-2 variety cumin plants at Sarla village after 1st foliar spray treatment (25 DAS) with 10% FOFCF, 5% ABFCF, 0.1% headline and control (D/W) at different time interval.

A: Induction of total protein concentration
B: Induction of total phenol concentration
C: Induction of reducing sugar concentration
D: Induction of non-reducing sugar concentration
Figure 90
Figure 91: Estimation of defense related enzymes in field grown GC-2 variety of cumin plants at Sarla village after 2nd foliar spray treatment (55 DAS) with 0.1 Headline, 10% FOFCF, 5% ABFCF and control (D/W) alone and in combination at different time interval.

A: Induction of phenylalanine ammonia lyase (PAL) activity

B: Induction of peroxidase (POX) activity

C: Induction of $\beta$-1,3 glucanase activity

D: Induction of polyphenol oxidase (PPO) activity
Figure 91
Figure 92: Estimation of defense related enzymes in field grown GC-2 variety of cumin plants at Sarla village after 2nd foliar spray treatment (55 DAS) with 0.1 Headline, 10% FOFCF, 5% ABFCF and control (D/W) alone and in combination at different time interval.

A: Induction of catalase activity

B: Induction of nitrate reductase (NRA) activity

C: Induction of nitrite reductase (NIR) activity
Figure 92
Figure 93: Estimation of defense related enzymes in field grown GC-2 variety of cumin plants at Sarla village after 2\textsuperscript{nd} foliar spray treatment (55 DAS) with 0.1 Headline, 10\% FOFCF, 5\% ABFCF and control (D/W) alone and in combination at different time interval.

A: Induction of total protein concentration

B: Induction of total phenol concentration

C: Induction of reducing sugar concentration

D: Induction of non-reducing sugar concentration
Figure 93
Figure 94: SDS-PAGE profiling of induced PR proteins from the FCF, headline treated and untreated plant samples after both treatments.

A: Protein profiling of plant samples collected after 1\textsuperscript{st} foliar spray treatment with various elicitors

Lane 1- Marker, Lane 2- Control, Lane 3- *Fusarium oxysporum f. sp. cumini* FCF, Lane 4- *Alternaria burnsii* FCF, Lane 5- Headline

B: Protein profiling of plant samples collected after 2\textsuperscript{nd} foliar spray treatment with various elicitors

Lane 1- Marker,
Lane 2- Control,
Lane 3- Headline

Lane 4- H(FO): 1\textsuperscript{st} spray with Headline followed by FOFCF in 2\textsuperscript{nd} spray,
Lane 5- H(AB): 1\textsuperscript{st} spray with Headline followed by ABFCF in 2\textsuperscript{nd} spray,
Lane 6- *Fusarium oxysporum f. sp. cumini* FCF,
Lane 7- FO(AB) FCF: 1\textsuperscript{st} spray with FOFCF followed by ABFCF in 2\textsuperscript{nd} spray,
Lane 8- *Alternaria burnsii* FCF,
Lane 9- AB(FO) FCF: 1\textsuperscript{st} spray with ABFCF followed by FOFCF in 2\textsuperscript{nd} spray

Induced additional protein bands
Figure 95: Effect of elicitors on physiological parameters of field grown GC-2 variety cumin plants at Sarla village.

A: Height and number of umbels in control and 0.1% headline, alone and in combination with FOFCF or ABFCF treated plants

B: Height and number of umbels in control and 10% FOFCF, FO(AB) FCF treated plants

C: Height and number of umbels in control and 5% ABFCF, AB(FO) FCF treated plants
Figure 96: Estimation of defense related enzymes in control, infected, nutrient deficient and elicitor treated plants.

A: Induction of PAL activity
B: Induction of POX activity
C: Induction of β-1,3-glucanase activity
D: Induction of PPO activity

Sample 1- Control
Sample 2- *F. oxysporum* infected plants
Sample 3- *A. burnsii* infected plants
Sample 4- nutrient deficient plants
Sample 5- 0.1% headline treated plants
Sample 6- 1.0% monitor treated plants
Sample 7- 10% FCF treated plants.
Figure 96
Figure 97: Estimation of defense related enzymes and total protein concentration in control, infected, nutrient deficient and elicitor treated plants.

A: Induction of catalase activity
B: Induction of nitrate reductase activity
C: Induction of nitrite reductase activity
D: Induction of total protein concentration

Sample 1- Control
Sample 2- *F. oxysporum* infected plants
Sample 3- *A. burnsii* infected plants
Sample 4- nutrient deficient plants
Sample 5- 0.1% headline treated plants
Sample 6- 1.0% monitor treated plants
Sample 7- 10% FCF treated plants.

Sample 1- Control
Sample 2- *F. oxysporum* infected plants
Sample 3- *A. burnsii* infected plants
Sample 4- nutrient deficient plants
Sample 5- 0.1% headline treated plants
Sample 6- 1.0% monitor treated plants
Sample 7- 10% FCF treated plants.
Figure 97
Figure 98: Estimation of total phenol, reducing sugar and non-reducing sugar in control, infected, nutrient deficient and elicitor treated plants.

A: Induction of total phenol concentration

B: Induction of reducing sugar concentration

C: Induction of non-reducing sugar concentration

Sample 1- Control

Sample 2 - *F. oxysporum* infected plants

Sample 3 - *A. burnsi* infected plants

Sample 4 - nutrient deficient plants

Sample 5 - 0.1% headline treated plants

Sample 6 - 1.0% monitor treated plants

Sample 7 - 10% FCF treated plants.
Figure 98
Figure 99: Bioassay of biocontrol agents and elicitors and phytoalexins isolated from treated and control plants against *Fusarium oxysporum* and *Alternaria burnsii*.

A: Appearance of zone of inhibition by the use of headline (100mg/ml) and monitor (100mg/ml) along with control against *F. oxysporum*

B: Appearance of zone of inhibition by the use of headline (100mg/ml) and monitor (mg/ml) along with control against *A. burnsii*

C: Appearance of zone of inhibition by the use of phytoalexins derived from cumin plant samples treated with 10% FOFCF, 0.1% headline, 1.0% monitor and control against *F. oxysporum*

D: Appearance of zone of inhibition by the use of phytoalexins derived from cumin plant samples treated with 5% ABFCF, 0.1% headline, 1.0% monitor and control against *A. burnsii*

E: Appearance of zone of inhibition by the use of phytoalexins derived from cumin plant samples treated with 10% FOFCF, 0.1% headline, 1.0% monitor and control against *F. oxysporum*

F: Appearance of zone of inhibition by the use of phytoalexins derived from cumin plant samples treated with 5% ABFCF, 0.1% headline, 1.0% monitor and control against *A. burnsii*
Figure 99