The experiment entitled “Biochemical characterization for induced systemic resistance by Bacillus antagonists against Fusarium wilt in cumin” was conducted at Department of Biochemistry, Junagadh Agricultural University, Junagadh during 2016-2017; the results are summarized and concluded in this chapter. The experimental material was comprised of 1 variety of cumin (GC-4) for biochemical analysis.

Fusarium wilt of cumin (Fusarium oxysporum) is a vascular disease caused by Fusarium oxysporum f. sp. cumin. The disease is widespread and causes substantial crop losses in many parts of the world. Annual yield losses due to Fusarium oxysporum f. sp. Cumin have been estimated to range from 10 to 15%. However, the disease can be controlled by use of resistant cultivars and adjustment of sowing dates. Several factors influence the efficacy of these management practices, including pathogenic variability in the fungus populations as well as abiotic factors such as temperature and moisture.

In this study an attempt has been made to understand the different variables which are directly or indirectly involved in a plant-pathogen interaction with following objectives.

1. To examine Fusarium wilt disease incidence and biocontrol efficiency of Bacillus antagonists.
2. To study phenols and phenolic profile during disease development stages and induced resistance by Bacillus antagonists.
3. To observe the antioxidative enzymes activities during disease development stages of Fusarium wilt of cumin.

Disease incidence

Maximum disease incidence was found (86.58%) in treatment- 6 (Pathogen (Fusarium oxysporum) infection (PI-P), at post-harvest stages (65 DAS) and lowest disease incidence (1.32%) was found in treatment- 5. Disease incidence was
maximum found in infected tissues of cumin as compared to non-infected tissues of cumin.

**Antioxidant activity**

Antioxidants are an important part of the defence system and help to cope with oxidative stress caused by reactive oxygen species. In cumin leaf and root tissues, antioxidant activity was found higher in treatment- 10 (PI-P + Nanoparticles of B. subtilis and B. prodigious) with reverse trend in their IC\textsubscript{50} values. Antioxidant activity was maximum found in non-infected plant tissue as compared to infected tissues of cumin.

**True protein**

In cumin leaf and root tissues, true protein content was (13.23 and 19.20 mg/g in fresh weight respectively) found higher in treatment- 2 (NI-P + B. subtilis) and treatment-9 (PI-P +B. subtilis + B. prodigios). True protein was maximum found in non-infected plant tissue as compared to infected tissues of cumin.

**Total phenol**

In cumin leaf tissues, total phenol was found higher in treatment- 11 (Absolute control (Fungicide carbendazim 0.02 %). In cumin root tissues, total phenol was found higher intreatment- 10 (PI-P + Nanoparticles of B. subtilis and B. prodigious,).

**Phenolic profiling by HPLC**

Phenolic compounds are the most important group implicated in both constitutive and induced resistance and also well-known anti-fungal, anti-bacterial and anti-viral compounds occurring in plants.

Total 15 phenolic acids were identified using HPLC. Cinnamic acid in cumin leaf tissue, was found highest in treatment- 2 (NI-P + B. subtilis) infection in plant (PI-P) and in cumin root tissue, was found highest in treatment- 4 (NI-P + B. subtilis+ B. prodigious). Highest cinnamic acid content was found in infected leaf and root tissues of cumin.
Caffeic acid in cumin leaf tissue was found maximum in treatment- 8 (PI-P + + *B. prodigiosus* (JND-KHCo-24-B)) and in cumin root tissue, was found highest in treatment-1 (Non-infested plant (NI-P)). Highest caffeic acid content was found in non-infected root and leaf tissues of cumin.

Salicylic acid in cumin leaf tissue, was found highest in treatment- 7 (PI-P + + *B. subtilis* (JSD-RSCu-8-D)). In cumin root tissue, was found highest in treatment- 4 (NI-P + *B. subtilis* + *B. prodigiosus*). Highest salicylic acid content was found in non-infected of root and highest in infected leaf tissues of cumin.

Gallic acid in cumin leaf tissue, was found highest in treatment- 10 (PI-P + Nanoparticles of *B. subtilis* and *B. prodigiosus*) and in cumin root tissue, was found highest in treatment-4 (NI-P + *B. subtilis* + *B. prodigiosus*). Highest gallic acid activity was found in infected root and leaf tissues of cumin.

Ferulic acid in cumin leaf tissue, was found highest in treatment- 6 (Pathogen (*Fusarium oxysporum*) infection (PI-P) and in cumin root tissue, was found highest in treatment- 5 (NI-P + Nanoparticles of *B. subtilis* + *B. prodigiosus*). Highest ferulic acid content was found in non-infected of leaf and highest in infected root tissues of cumin.

Quercetin acid in cumin leaf tissue, was found highest in treatment- 1 (Non-infected plants (NI-P)) where as in cumin root tissue, it was found highest in treatment- 4 (NI-P + *B. subtilis* + *B. prodigiosus*). Highest quercetin acid content was found in infected of leaf and highest in non-infected root tissues of cumin.

Catechol in cumin leaf tissue, was found highest in treatment- 5 (NI-P + Nanoparticles of *B. subtilis* + *B. prodigiosus*) where as in cumin root tissue, was found highest in treatment- 8 (PI-P + + *B. prodigiosus* (JND-KHCo-24-B)). Highest catechol content was found in non-infected of leaf and highest in infected root tissues of cumin.

Chlorogenic acid in cumin leaf and root tissue, was found highest in treatment- 3 (NI-P + *B. prodigiosus*) and treatment-1 (Non-infected plants (NI-P)). Highest chlorogenic acid was found in non-infected leaf and root tissues of cumin.
Summary and Conclusion

Coumaric acid in cumin leaf tissue was found highest in treatment- 9 (PI-P + B. subtilis + B. prodigious). In cumin root tissue, was found highest in treatment- 2 (NI-P + B. subtilis). Highest coumaric acid was found in infected leaf and root tissues of cumin.

Syringic acid in cumin leaf tissue, was found highest in treatment- 5 (NI-P + Nanoparticles of B. subtilis + B. prodigious). In cumin root tissue, was found highest in treatment- 4 (NI-P + B. subtilis+ B. prodigious). Highest syringic acid activity was found in non-infected leaf and root tissues of cumin.

Kaempferol acid in cumin leaf tissue, was found highest in treatment- 11 (Absolute control (Fungicide carbendazim 0.02 %). In cumin root tissue, was found highest in treatment- 1 (Non-infected plants (NI-P)).Highest kaempferol acid activity was found in infected leaf and highest in non-infected root tissues of cumin.

Vanillic acid in cumin leaf tissue, was found highest in treatment- 6 (Pathogen (Fusarium oxysporum) infection (PI-P). In cumin root tissue, was found highest in treatment- 4 (NI-P + B. subtilis+ B. prodigious).Highest vanillic acid activity was found in infected leaf and root tissues of cumin.

Catechin acid in cumin leaf tissue, was found highest in treatment- 5 (NI-P + Nanoparticles of B. subtilis + B. prodigious).In cumin root tissue, was found highest in treatment- 2 (NI-P + B. subtilis). Highest catechin acid activity was found in infected leaf and highest in non-infected root tissues of cumin.

Epicatechin acid in cumin leaf tissue, was found highest in treatment- 11 (Absolute control (Fungicide carbendazim 0.02 %). In cumin root tissue, was found highest in treatment- 1 (Non-infested plant (NI-P). Highest epicatechin acid was found in non-infected leaf and root tissues of cumin.

Epigallocatechin acid in cumin leaf tissue, was found highest in treatment- 6 (Pathogen (Fusarium oxysporum) infection (PI-P). In cumin root tissue, was found highest in treatment- 8 (PI-P + + B. prodigious (JND-KHCo-24-B).Highest epigallocatechin acid was found in infected leaf and root tissues of cumin. Whereas lower concentration observed during the per-infection stage of disease development. This trend was observed same for all phenolics compounds.
Summary and Conclusion

**Guaiacol peroxidase**

In cumin leaf tissues, guaiacol peroxidase activity was found higher in treatment- 7 (PI-P + + B. subtilis (JSD-RSCu-8-D) and treatment- 8(PI-P + + B. prodigiosus (JND-KHCo-24-B)). In cumin root tissues, guaiacol peroxidase activity was found higher in treatment- 10 (PI-P + Nanoparticles of B. subtilis and B. prodigious).Highest Guaiacol peroxidase activity was found in non-infected leaf and highest in infected root tissues of cumin. As disease development stages increasing the guaiacol peroxidase activity increased in both leaf and root tissues of cumin.In guaiacol peroxidase leaf specific activity and root specific activity were highest in treatment- 4 (NI-P + B. subtilis+ B. prodigious).As disease development stage increase the GPX specific activity was increases.

**Ascorbate peroxidase**

In cumin leaf tissues, ascorbate peroxidase was found higher in treatment- 7 (PI-P + + B. subtilis (JSD-RSCu-8-D)). In cumin root tissues, ascorbate peroxidase was found in treatment- 11 (Absolute control (Fungicide carbendazim 0.02 %). Highest Ascorbate peroxidase activity was found in non-infected leaf and highest in infected root tissues of cumin. Ascorbate peroxidase activity increasing as increasing at disease development stages in both leaf and root tissues of cumin.In ascorbate peroxidase leaf specific activity was highest in treatment- 6 (Pathogen (Fusarium oxysporum) infection (PI-P)) and ascorbate peroxidase root specific activity was highest in treatment- 11 (Absolute control (Fungicide carbendazim 0.02 %)).

**Polyphenol oxidase**

In cumin leaf and root tissues, polyphenol oxidase was found higher in treatment- 10 (PI-P + Nanoparticles of B. subtilis and B. prodigious) and treatment-4(NI-P + B. subtilis+ B. prodigious.). Polyphenol oxidase was maximum found in infected plant tissue as compared to non-infected tissues of cumin. As disease development stages increasing the polyphenol oxidase in both leaf and root of cumin.In polyphenol oxidase leaf specific activity was highest in treatment- 10 (PI-P + Nanoparticles of B. subtilis and B. prodigious)) and polyphenol oxidase root specific activity was highest in treatment- 4 (NI-P + B. subtilis+ B. prodigious).
Catalase

In cumin leaf tissues, catalase was found higher in treatment- 1 (Non-infected plants (NI-P)). In cumin root tissues, catalase was found higher in treatment- 11 (Absolute control (Fungicide carbendazim 0.02 %)). Highest catalase activity was found in non-infected leaf tissue and highest in infected root tissues of cumin. Catalase activity significant decreased as disease development stages increased in both leaf and root tissues of cumin. In catalase leaf specific activity was highest in treatment- 1 (Non-infected plants (NI-P)), and root specific activity was highest in treatment- 11 (Absolute control (Fungicide carbendazim 0.02 %)).

CONCLUSIONS

The present investigation indicates, the phenolic content was help to distinguishes difference in different bio-agent treatment during all stages of disease development. The phenol profiling of cumin variety also poorly distinguishes difference between different bio-agent treatment and disease development stages.

Based on biochemical study that revealed infected tissue of cumin variety showed high in their antioxidant enzyme activity and also they were differ in their interaction observed during the infection stages of disease development.

FUTURE RESEARCH NEEDS

- More detail Biochemical study on disease control mechanisms, will be taken up for clear conclusion.

- Much more needs to be done for an effective understanding of both host pathogen and their interaction.