Summary

This work presents a comparative study between the antimicrobial properties of chemically synthesized bioactive benzoxazinone compounds and the ethanolic extracts of carnation stems (*Dianthus caryophyllus*); as ornamental plants, of widely grown American cultivar, namely, cv. Madam collect, naturally infected by the fungal pathogen *Fusarium oxysporum* (*FO*) which contains the phytoalexin dianthalexine besides other phytoalexins that were previously reported to contribute to the resistance of carnation against this pathogenic fungus.

The thesis consists of three parts (chemically synthesized benzoxazinones; biosynthesis of benzoxazinone (phytoalexins, dianthalexine); antimicrobial activities of ethanolic extracts of carnation stems and chemically synthesized benzoxazinones.

Each part is divided into three chapters (introduction, results and discussion, and experimental part).

The first part

It deals with the synthesis of 2-phenyl-4*H*-3,1-benzoxazin-4-one compound, in addition to study its behavior towards some interesting nitrogen nucleophiles. Moreover, synthesis of benzoxazinone derivatives was carried out. Furthermore, elucidation of the structure of all synthesizable compounds by IR and Mass spectral data was discussed. This part consists of three chapters:

Chapter one, introduction, provides an overview of the importance of 4*H*-3,1-benzoxazin-4-one class and describes recent works in the field of synthesis of derivatives of 4*H*-3,1-benzoxazin-4-ones. Furthermore, the reaction of 4*H*-3,1-benzoxazin-4-ones is described.

Chapter two, results and discussion, it focuses on the obtained results and discusses these results. The obtained results can be summarized as:

- A series of benzoxazinone and quinazolinone derivatives **III**-**XXV** are obtained.

- Elucidation of the structure of all synthesizable compounds by IR and Mass spectral data is discussed. These data are evaluated to confirm the chemical structure of all synthesized compounds. Prediction of some reaction mechanisms is discussed.

Chapter three, experimental section, it demonstrates the procedure of the preparation of all chemically synthesized compounds.

The second part

This part deals with the extraction of phytoalexin dianthalexine from the artificially infected stems of two widely grown cultivars, namely, Egyptian carnation cv. Balady and American carnation cv. Madam collect with *FOD*, cut out after two and eight weeks. Moreover, the extraction of phytoalexin dianthalexine from the naturally *FO* infected stems of American carnation cv. Madam Collect (from the infected stems and the recovered ones) was done. Also, separation and detection of dianthalexine by high pressure liquid chromatography (HPLC) was carried out. This part is divided into three chapters:

Chapter one, introduction, which summaries the importance of carnation based on the outline of this work. Moreover, other research studies related to the present work were discussed. In addition to, a brief notes that focused on the resistance of plants against pathogens was presented.

Chapter two, results and discussion, it gives the details of the obtained results and discusses the significance of these results. The obtained results can be summarized as:

- In artificially infected carnation stems, the highest concentration of phytoalexin dianthalexine was found in the Egyptian carnation cv. Balady

cut out after eight weeks, although, those samples showed yellowing and wilting symptoms.

- In naturally infected stems of American carnation, infected stem samples accumulated dianthalexine in higher concentration than the recovery stem samples of the same plant.

- Identification of phytoalexin dianthalexine peak using high performance liquid chromatography (HPLC).

Based on these obtained results, the predication of possible mechanisms of the resistance of carnation against *FOD* was highlighted and discussed.

Chapter three, experimental section, it demonstrates the method of extracting of phytoalexins from infected carnation stems. In addition, the technique of identifying the phytoalexin dianthalexine by high performance liquid chromatography (HPLC) was described.

The third part

This part deals with the main scope of this study. This scope focuses on finding sources of bioactive compounds such as the natural plant – derived products (ethanolic carnation extracts that contained phytoalexin dianthalexine). Moreover, the *in-vitro* biological activities of synthesized compounds were studied. Based on the economically importance of carnation, the fighting of carnation pathogen i.e. *FOD* was discussed. The third part is divided into two main sections (sections 1 & 2):

- **Part (1)** deals with the comparison between the antimicrobial activities of the ethanolic extracts of the naturally infected American carnation cv. Madam collect and 2-phenyl-4*H*-3,1-benzoxazin-4-one **III,** and 2-phenyl-3-aminoquinazolinone **XII** compounds towards the human and plant pathogenic bacteria and fungi. This part is divided into three chapters (introduction, results and discussion, experimental section):

- Introduction, it summarizes the hazardous of pathogenic fungi and bacteria on human health and its impact on economy.

- The obtained results can be summarized as:

- In antifungal activities test, the tested samples of the extracts of the naturally infected stems of American carnation plants cv. Madam collect, sampled from the infected stems and from the recovered ones, and of the chemically synthesized 2-Phenyl-3,1-benzoxazin-4-one and 2-phenyl-3-amino-4(3*H*)quinazolinone compounds did not exhibit activities against *Aspergillus fumigatus*, *Candida ablicans* and *Fusarium monliforme*.

- *FOD* had only been moderately affected by the ethanolic extracts of both of the infected and the recovered ones at concentration of 10 µg/ml. Whereas , 2-Phenyl-3,1-benzoxazin-4-one, at a concentration of 5 µg/ml was moderately active against *FOD* since a slight growth and an irregular pattern of fungal colonies growth have been observed.

- In antibacterial activities, these tested samples did not exhibit activities against the two Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*.

- The tested samples exhibited close activities against *Staphylococcus aureus* and *Bacillus subtilis* at concentrations of 5 and 10µg/ml.

- 2-phenyl-4*H*-3,1-benzoxazin-4-one compound is the most active tested compound against *S aureus* at concentrations of 5 and 10µg/ml.

- 2-phenyl-4*H*-3,1-benzoxazin-4-one compound is also the most active tested compound against *B subtilis* at concentration of 5 µg/ml. While, the extract of the infected site of the American carnation, naturally infected with *FO* was the most active tested sample against *B subtilis* at concentration of 10µg/ml.

- The relationship between the chemical structure and the antimicrobial activities of these tested samples was proposed and discussed.

- The experimental section, describes the method of diffusion agar plate test for the evaluation of antimicrobial activities of the tested samples. This section was summarized as follows:

- The comparison of antifungal activities of the tested samples of the extracts of the naturally infected American carnation plants cv. Madam collect, sampled from the infected stem and from the recovered ones, and of the chemically synthesized 2-Phenyl-3,1-benzoxazin-4-one and 2-phenyl-3-amino-4(3*H*)quinazolinone compounds against the fungal human pathogens *A fumigatus* and *C ablicans* was studied. In addition, the two plant fungal pathogens i.e. *F monliforme* and *FOD* were also tested.

- The comparison of antibacterial activities of the tested samples against the two Gram positive bacteria *S aureus* and *B subtilis* as human pathogens was studied. In addition, the two Gram negative bacteria *E coli* and *P aeruginosa* also were used as human pathogens.

- Statistical studies of the antimicrobial bioassay were carried out.

Part (2), deals with the antimicrobial activities of all of the chemically synthesized compounds **IV**- **XXV**, except 2-phenyl-3-amino-4(3*H*)quinazolinone **XII** against *FOD* as carnation fungal pathogen. Moreover, the antimicrobial activities of these compounds against the Gram negative bacterium i.e. *Agrobacterium tumefaciens* as peach pathogen was studied. Furthermore, their activities against the Gram positive bacterium *B subtilis* as biocontrol agent were studied. This part is divided into three chapters:

- Introduction, provides an outline of the harmful impact of these pathogens on human and plant health.

- Results and discussion present the obtained results and discuss its significance.

- The obtained results can be summarized as follows:

- In the antifungal bioassay,2-[2-phenylcarbonylaminophenylcarbonyl) amino benzoic acid **VII** compound, in comparison of the test compounds, was the most active test compound against the carnation pathogenic fungus *FOD*.

- In the antibacterial bioassay, 2-{[2-(benzoylamino) benzoyl] amino} acetic acid **IV a** compound was the most active compound tested against the Gram negative bacterium *A tumefuaciens*. While, the most active compound against the Gram positive bacterium *Bacillus subtilis* as biocontrol agent against phytopathogenic microorganisms was 2-[2(phenylcarbonylaminophenylcarbonyl] amino benzoic acid compound.

- Prediction of the structure-activities relation (SAR) of these tested compounds was discussed.

- The experimental work describes the diffusion agar plate test used for evaluating the antimicrobial activities of all the synthesized compounds **IV**-**XXV**, except 2-phenyl-3-amino-4(3*H*)quinazolinone **XII**. It is summarized as follows:

- The comparison of antifungal activities of the test compounds against the pathogenic fungus *FOD* as a carnation plant pathogen was studied.

- The comparison of antibacterial activities of the test compounds against the Gram negative bacterium *A tumefuaciens* as peach plant pathogen was also studied. In addition, the Gram positive bacterium *Bacillus subtilis*, as biocontrol agent against phytopathogenic microorganisms, was studied.

- Statistical studies of the antimicrobial test were carried out.

Conclusion, naturally and artificially infected carnation plants which showed disease symptoms or symptomless can act as natural source of potentially benzoxazinone derived phytoalexin like dianthalexine and other phytoalexins. Furthermore, an adding value would be in recycling the wasted and the infected plants and utilize them as a potential

antimicrobial source for implementing potential industry development strategies, for the welfare of all live stock in Egypt and the whole world. In turn, these strategies would benefit eliminating the poverty especially in Egypt and the developing countries else where in the globe, in different biotechnological sectors such as food, drugs and biocides industries. Besides, it is safer to our environment, and cheaper than the chemically prepared compounds.