DISCUSSION
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Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural resources. Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents (Racio et al., 1989). Emergence of pathogenic microorganisms that are resistant/multi-resistant to major classes of antibiotics have increased in recent years due to indiscriminate use of synthetic antimicrobial drugs (Karaman et al., 2003). In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics (such as hypersensitivity, allergic reactions, immunosuppression etc.) and are major burning global issues in treating infectious diseases. Although pharmaceutical industries had produced considerable number of commercial antibiotics time to time but resistance in pathogens towards these drugs too has increased at high rate and multi drug resistant microorganisms have exacerbated the situation (Nino et al., 2006). In the present scenario, there is an urgent and continuous need of exploration and development of cheaper, effective new plant based drugs with better bioactive potentiality and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines (Sharma B, Kumar P., 2008-09).

Plants continue to be a major source of medicines, as they have been through out human history. It is estimated that roughly 1500 plants species in Ayurveda and 1200 plant species in Siddha have been used for drug preparation, nearly 80% of the world population rely on traditional medicines for primary health care, most of which involve the use of plant extract as traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed.

Antimicrobials of plant origin have enormous therapeutic potentiality and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics (Iwu at al., 1999). Positive response of plant based drugs (less/no side effects) might lies in the structure of the natural products which reacts with toxins and/or pathogens in such a way that less harm is done to other important molecules or physiology of host. It is because of this reason that drug designing studies nowadays have come up as new field of research.

In this thesis the authoress has depicted a crop protective formulation for controlling infestation caused by *Fusarium oxysporum* in *Pisum sativum* L. plants by a newly isolated flavone (3,7-dihydroxy 3′,4′ orthodihydroxy flavone) from *Clitoria ternatea* L.

Chapter 1 entails screening of antimicrobial action of 50% aqueous ethanolic extract of *C. ternatea* L. by agar cup diffusion method. Antibacterial effect was tested against *Serratia marcescens* (7298), *Erwinia herbicola* (3609), *Xanthomonas sp.* (7444), *Arthrobacter*
chlorophenolicus (3706). Antifungal effect was tested against Botrytis cineria (MTCC/067/4260, code 357), Fusarium oxysporum, Rhizoctonia solani, Aspergillus flavus. (Table 1.1, 1.2 respectively).

Activity was found in 50% aq. ethanolic leaf extract of C. ternatea L. against Arthrobacter chlorophenolicus.

Antifungal activity of 50% aq. Ethanolic extract was detected against Fusarium oxysporum ciceri. In our laboratory, a management protocol for arresting fungal damage in Cicer arietinum L. by another newly isolated flavone (7-hydroxyflavone) from Cassia fistula L. was formulated. In this thesis, our aim was to evaluate whether F. oxysporum ciceri can also act as a pathogen of P. sativum L. and if so to raise an antifungal formulation in that respect. (Dutta T. and Chatterjee, P., 2008).

Successively the bioactive 50% aq.ethanolic extract was subjected to column chromatographic separation (Table 1.8 vide page no.66). Each residual fraction obtained from the column chromatography was again subjected to antifungal bioassay (table 1.9, vide page.66). Results indicated antifungal potentiality was present in the chloroform:carbinol fraction 4. Bioactivity was tested against the same fungus. (MIC value 35 mg/ml, table 1.10).

Antitumour activity was performed by brium shrimp by Mayer et al (ref) an inexpensive cost effective internationally accepted protocol for determining antitumour action. Table 1.6, 1.7, page 64,67. LC 50=0.32532mg/l (28121.366) mg/l.

So, results of chapter 1 depicts antifungal, antibacterial and antitumour activity in the tested 50% aq.ethanolic extract of C. ternatea L.

However, the author in this particular thesis gives a detailed emphasis to evaluate the antitumour efficiency of C. ternatea L. and subsequently to ensure a nontoxic, ecofriendly management protocol in relation to infestation caused by F. oxysporum ciceri in pea plants.

Chapter 2 entales purification and identification of the bioactive antifungal principle of C. ternatea L. The principle bioactive sample obtained from chloroform: carbinol fr.4 had a melting point 245⁰C. The sample was homogeneous, Rf values of 0.994 in TBA (butanol: glacial acetic acid: water = 3:1:1) and 0.882 in HOAc (glacial acetic acid: water = 3:17). The spot appeared with a violet fluorescence in UV light and dark mauve when treated with NH₃ under UV light. This sample was subjected to uv spectroscopic analysis. This sample had melting point at 245.⁰C. The spot appeared with a violet fluorescence in UV light and dark mauve when treated with NH₃ under UV light indicating flavonoid nature of the compound. This sample was then taken up for further uv spectral analysis following Mabry (ref) for their chemical characterization.
**Chapter 3** incorporates in vitro assessment of antifungal activity of Flavone with seeds of *P. sativum* L. against *F. oxysporum ciceri* done with different experimental set up of *P. sativum* L.

Germination percentage and TTC stainability of the seed sets as designated as page no. 59 are depicted in fig 3.1, 3.2 and table 3.1, 3.2. Results indicated that *P. sativum* L. seeds infested with *Fusarium oxysporum ciceri* had very less germination percentage as well as TTC stainability during 24, 48, 72 hours intervals. In all the cases, presoaking of the seeds (3 hrs) with MIC value (35 mg/ml) increased both the parameters. The promotive effect of germination percentage and TTC stainability with flavone was higher than with gresiofulvin. Results also indicated that isolated F was nontoxic towards healthy seeds.

Table 3.3 to 3.5 and fig. 3.3 to 3.5 depicts total sugar, protein and amino acid content. In these three cases, the reduction in the contents in the considered component caused by *Fusarium* was impaired with administration of isolated flavone. From the tables it was also evident that total sugar, protein and amino acid contents increased by presoaking the seeds with the flavone. Antifungal efficiency of F was greater than gresiofulvin and it was also interesting to note that F had a promotive effect over control. So the F may be cited both as non toxic antifungal compound as well as a promoter.

From the tables 3.6 and fig. 3.6(a), it is evident that effect of F on DNA content does not focus major attention however, its effect on RNA content is better (fig.3.6.b).

From various reports like (ref) it is clear that pathogen infestation causes increase in phenol content. Our results also give us the similar inference where there was an increase in total phenol content by fungal infestation. Table 3.7 and figure 3.7 also indicates this increase and this increase was lowered by the isolated F treatment (table 3.8 fig.3.8).

Proline content increases in fungus infested seeds whereas it very much decreases when the fungal infested seeds were administered with F indicating the isolated sample was a stress reliever (table 3.9, fig. 3.9).

In addition to the above quantitative estimation of the above component chapter 3 also furnishes a detailed comparison of some important metabolic and scavenging enzymes. Fig. 3.10, 3.11 table 3.10, 3.11 illustrates activities of amylase, protease. Results indicate retardation of the activities upon fungal infestation, acceleration of the same enzymes with F. Also as discussed earlier, page 110, 112, F also showed a promotive effect on amylase and protease. It may also be noted as gresiofulvin is less effective than the isolated F of *C. ternatea* L.

It is widely known that infestation leads to acceleration of respiratory enzymes. This concept has also been supported by our results depicted in table3.14, 3.16, 3.17 and fig 3.14, 3.16, 3.17. The studied respiratory enzymes were total dehydrogenase, malate dehydrogenase and succinate dehydrogenase. In all three cases, fungal infestation lead to increase in all respiratory enzymes, presoaking seeds with F lowered the activities. The authoress here wants to say that the selection of two specific respiratory enzyme was done at random
keeping in mind the fact that the coenzyme partners of these two enzymes were different (NAD for malate dehydrogenase and FAD for succinate dehydrogenase).

Table 3.12, 3.13, 3.15, 3.18, 3.19 and fig. 3.12, 3.13, 3.15, 3.18, 3.19 evaluates the comparative activities of scavenging enzymes ie. super oxide dismutase, catalase, Peroxidase, catechol oxidase, laccase. Table 3.12, 3.13, 3.15, 3.18, 3.19 indicated an increase in activity of these enzymes in fungus infested seeds, reduction in the enzyme activities by presoaking the seeds with 3,7-dihydroxy 3',4' orthodihydroxy flavone. Results also indicate efficiency of the isolated F to be more than that of gresiofulvin in relation to the activities of the stress enzymes.

Chapter 4

From previous chapters, we established antifungal effect of \textit{Fusarium oxyporum ciceri} infesting seeds of \textit{P. sativum} L. This chapter 4 entails evaluation of the isolated bioactive flavone in terms of its antifungal action against \textit{Fusarium oxysporum ciceri} causing damage to \textit{P. sativum} L. seedlings (studies incorporated on 21\textsuperscript{st} day, extending up to 63\textsuperscript{rd} days i.e. from the vegetative to the reproductive stages of the plants).

During the vegetative stage some morphological parameters as well as biochemical parameters were studied. Table 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7 incorporate comparative study of root length, shoot length, internode length, leaf length, petiole, leaflet length, and pod length of the different experimental sets observed on the 21\textsuperscript{st} day of the seedling growth. In all the cases it was noticed that fungal infestation by \textit{Fusarium oxysporum ciceri} caused retardation of studied parameters, whereas treatment with 3,7-dihydroxy 3',4' orthodihydroxy flavone had a promotive effect. This promotive effect with F was greater than that of the gresiofulvin and the flavone was also a promoter of shoot length (fig. 4.3, 4.4), internode length (fig. 4.8), leaf length (fig. 4.9), petiole (fig. 4.10, 4.11), leaflet length (fig. 4.13) and pod length (fig. 4.14) over healthy set.

Table 4.9, 4.10, 4.11 depicts quantitative estimation of chlorophyll a, chlorophyll b and total chlorophyll content on 21\textsuperscript{st} day of all the experimental sets (fig. 4.16, 4.17, 4.18). However, total chlorophyll, chlorophyll a and chlorophyll b content increased upon fungal infestation and the flavone successfully decreased the contents. Table 4.12, fig. 4.19 shows decrease in carotenoid content during fungal infestation and it was increased by the F. Efficiency of the flavone was greater than that of gresiofulvin.

Table 4.13, 4.14, 4.15, 4.16, 4.18 evaluates total sugar, total reducing sugar, total non reducing sugar, total amino acid and total protein contents. In each case \textit{F. oxysporum ciceri} inhibited content, administered 3,7-dihydroxy 3',4' orthodihydroxy flavone increased the contents with an efficiency with more than that of gresiofulvin.

Picture of nucleic acid and total lipid content vide table 4.17 may not be mentioned.
From table 4.21, 4.24, it may be found that fungal infestation increased the total phenol and proline contents. Total phenol, other phenol (table 4.23) and proline contents increased in fungal infestation; however the picture of o-dihydric phenol was opposite (table 4.22).

Table 4.25, 4.26, 4.27, 4.28, 4.29 and 4.30 represent a comparative analysis of some enzyme activities in the experimental sets like dehydrogenase, peroxidase, catalase, super oxide dismutase, laccase and catechol oxidase. Infestation by *Fusarium* increased the respiratory enzyme dehydrogenase as well as the scavenging enzymes. Administration of F lowered the enzyme activities. This is in concurrence with the concept that infestation leads to acceleration of respiratory and stress enzymes.

Table 4.31, 4.32, 4.33, 4.34, 4.35, 4.36 incorporates comparative study of root length, shoot length, internode length, leaf length, leaflet length, no. of leaves of the different experimental sets observed on the 42nd and 63rd day of the seedling growth. Table 4.37, 4.38, 4.39, 4.40 evaluates comparative account of the no. of flowers/plant, no. of pods/plant, pod size and no. of seeds/pod of the experimental sets on 42nd and 63rd day of the plants. In all the cases *Fusarium oxysporum ciceri* caused inhibition. It was found that fungicide treated fungus infested sets failed to develop any pods as well as seeds in the plants. Administration of F exerted promotion with efficiency more than gresiofulvin. Moreover the promotive effect of the isolated F was greater over healthy as seen before vide page 145, 150, 154, 155.

Table 4.44, 4.42, 4.43 depicts total chlorophyll, chlorophyll a and chlorophyll b content. The highlighting features of these tables suggests a promotive effect of F over healthy whereas the picture of carotenoid content of the seedlings were lowered by *Fusarium* infestation, increased with administration with the F. antifungal efficiency of the F was greater than gresiofulvin. Here also the F increased carotenoid content of the healthy plants (table 4.45).

Table 4.46, 4.47, 4.48, 4.49, 4.51, 4.53 shows total sugar, total reducing sugar, non reducing sugar, amino acid, protein and lipid contents. In all the experimental sets, it was found that fungal infestation lowered the individual content, administration of the F increased the content more efficiently than gresiofulvin. As noted earlier, the isolated F has a promotive effect on healthy plants. Increase in protein content with administration with 3,7-dihydroxy 3’,4’ orthodihydroxy flavone in normal healthy plants as well as in fungus infested plants is a promising indicator for improving seed quality.

Picture of nucleic acid vide table 4.50 exhibited that fungal infestation lowered the individual content, administration of the F increased the content more efficiently than gresiofulvin. As noted earlier, the isolated F has a promotive effect on healthy plants.

From table 4.54, 4.55, 4.56, 4.57 it may be found that fungal infestation increased the total phenol, ortho dihydric phenol, other phenols and proline contents. Total phenol, other phenol (table 4.23) and proline contents increased in fungal infestation but administration of F lowered the content levels.

Table 4.58, 4.59, 4.27, 4.60, 4.61, 4.62 and 4.63 represent a comparative analysis of some enzyme activities in the experimental sets like dehydrogenase, peroxidase, catalase, super
oxide dismutase, laccase and catechol oxidase. Infestation by *Fusarium* increased the respiratory enzyme dehydrogenase as well as the scavenging enzymes. Administration of **3,7-dihydroxy 3',4' orthodihydroxy flavone** lowered the enzyme activities. This is in concurrence with the concept that infestation leads to acceleration of respiratory and stress enzymes. The isolated flavone was stress reliever.

Analysis of gel documentation revealed few observations. The alterations in the protein banding patterns in SDS gel in both seed and leaf in the **3,7-dihydroxy 3',4' orthodihydroxy flavone** treated sets may be involved in the recovery of the physiological and metabolic state caused by the fungal infestation. **3,7-dihydroxy 3',4' orthodihydroxy flavone** induced production of few new protein molecules of low and medium molecular weight compared to that of healthy and fungus infested sets.

Fig. 3.24, 3.25 3.26, 3.27, 3.28 represents banding patterns of the enzymes peroxidase, catalase, super oxide dismutase, catechol oxidase, malate dehydrogenase of seeds of in vitro study.

Fig. 4.101, 4.102, 4.103, 4.104, 4.105, 4.106 represent the enzyme banding patterns of peroxidase, catalase, super oxide dismutase, catechol oxidase, malate dehydrogenase, Poly Phenol Oxidase (PPO) of leaf in 63\textsuperscript{rd} day of treatment where as fig. 4.107, 4.108, 4.109, 4.110 shows enzyme activities of catalase, peroxidase, malate dehydrogenase, catechol oxidase of seeds of the field plant products expressed in terms of the band intensities. The enzyme band intensities developed in the native gels of both seeds and leaf of various enzymes were similar to the data calculated in the quantitative estimations of the enzymes activities (tables 3.12, 3.13, 3.15, 3.18, 3.19, 4.25, 4.26, 4.27, 4.28, 4.29, 4.30, 4.58, 4.59, 4.27, 4.60, 4.61, 4.62 and 4.63).

So, it can be concluded that **3,7-dihydroxy 3',4' orthodihydroxy flavone** can be recomended as a non toxic antifungal crop protectant and also a growth promoter in the present study.