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Original Research Article

Meteorological parameters of fungi and its antimicrobial activity in hospital environment

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Keywords

Penicillium spp, Antimicrobial activity, Meteorological parameters, Pearson's co-efficient.

A B S T R A C T

The atmospheric fungal spores are an ever-present component and well known particulate matter to trigger asthma and hay fever symptoms in atopic individuals. The hospital atmosphere near Chennai Central, Chennai, Tamilnadu has been monitored for airborne fungal spores at several sampling stations by Petri-plate settlement method. In qualitative and quantitative way, fungal spores considerably varied from season to season. The proposed study involved the examination of the antimicrobial activity of *Penicillium* spp and correlated with meteorological data recorded during the study period. The bioactive compounds of *Penicillium* spp of hospital environment at different sites of different seasons were extracted and then subjected for its antimicrobial activity study with different bacterial pathogens. The activity was also compared using different antibiotic discs (Tetracycline, Vancomycin, Ampicillin, Amoxicillin and Penicillin-G). The *Penicillium* spp isolated from hospital environment during winter season showed good activity against the pathogens than the other seasons.

Introduction

Microorganisms are ubiquitous in the environment that can be beneficial or harmful or in apparent with regard to human measure or observation. Environmental pollution broadly refers to the presence of undesirable substances in the environment which are harmful to man and other organisms. The pollutants are chemical, biological and physical in nature. Bacteria have developed resistance to all different classes of antibiotics and its activity varies based on the climatic conditions. Hospital environments that require great care in terms of the environmental monitoring is now critical (Jose et al., 2005; Hidron et al., 2008). The beneficial effects of microbes derive from their metabolic activities in the environment, use in food production, biotechnological processes, for the treatment and prevention of infectious diseases (Todar, 2011). Antibiotics are substances produced by microorganisms that kill or inhibit other microbes which are used in the treatment of infectious diseases (Chikere et al., 2008). Many fungi also produce antibiotic substances, which are now widely used to control diseases in human and animal populations. Hospitals have become a breeding ground for antibiotic resistant bacteria, where meteorological parameters play a very important role. Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century. Fungi are of fundamental importance in the terrestrial environment, dominant in soil and air can comprise the largest pool of biomass. Extracting bioactive compounds or biosynthetic methods by using microorganisms are ecofriendly in nature and it has been investigated as an alternative to chemical and physical ones (Sastry et al., 2003). The present study was carried out to find out the antimicrobial activity of bioactive compounds extracted from *Penicillium* spp isolated from different sites and seasons against various human pathogens.

Materials and Methods

Study sites

Chennai is a metropolitan city and the State Capital of Tamilnadu, where floating population increases day by day and Chennai Central (Railway station) is the busiest place in the Southern Railway. More than two to three lakhs of people generally use this place every day as a floating population. Chennai is well known for its IT hub, expanding industries and has a moderate climate. Usually the temperature varies from 28 to 38°C throughout the year, with heat waves in summer season. The coolest month is December and January with low temperature and the hottest month is April and May with high temperature. Winter temperatures rarely drop below 25°C and summer temperatures seldom exceed 40°C. Chennai receives only re-treating monsoon during October to December.

Air Samplings

The samples were collected from in and around hospital environment near Chennai Central, Chennai, by exposing the Sabouraud's dextrose agar media plate incorporated with antibiotic (50mg$^{1)}$ by settle plate technique. The plates were exposed to air inside the hospital 5ft from the ground and 1km distance from the hospital for 5 minutes just by keeping the media plates 2m above the ground level to avoid dusting. The plates were incubated at 25±3^{o}C temperature for 5-7 days. Fungal colonies developed in plates were counted for individual species and to get the total number CFUs. Microscopic slides stained with lacto phenol cotton blue were prepared from each CFUs and observed microscopically to identify them upto species level. The colony forming units (CFUs) that could not be identified directly from plates were sub cultured in PDA/SDA/CDA media again and identified later on. The laboratory experience and taxonomic literature were employed to identify the fungal taxa. Annual and monthly percentage occurrence of individual fungus was determined and the antimicrobial activities of allergenic fungi were studied. Antibacterial activities of the bioactive compounds produced from *Penicillium* spp was given only in this paper.

Extraction of Bioactive compounds:

Penicillium spp were cultured in the Sabouraud's Dextrose Broth and allowed to grow upto 25 days. After the change in color of the broth, it was decanted slowly and subjected to the addition of ethyl acetate and chloroform at equal ratio to the filtrate in a separating funnel. With continuation of shaking for five minutes, the separating funnel was kept at stand still condition and the bioactive compounds were collected slowly at the lower part in a separate conical flask. The separated compounds were kept in the rotary evaporator for extracting the bioactive compounds.

Antimicrobial Activity Test

Disc diffusion method was used for checking the antimicrobial activity of *Penicillium* spp against bacterial pathogens like *Pseudomonas aeruginosa, S. epidermidis, Bacillus cereus, E. coli,* and *Staphylococcus aureus*.. The zone of inhibition were measured and compared with the control in its raw form and with the different antibiotic disc to confirm their activity ability.

Results and Discussion

Among the different sampling techniques for aeromycological studies, gravity settling of spores on culture media is one of the widely used techniques by different workers (Hedayati et al., 2005). The present study rightly used this technique expressing the results only qualitatively. Out of the isolated fungal species, the present study focused on the common *Penicillium* spp collected from different sites and seasons. Outdoor environment contributed more spore profile in comparison to indoor environment of the hospital (Nanda et al., 2000). In the comparative analysis among members of fungal species, *Penicillium citrinum, P. chrysogenum* and *P. oxalicum* contributed the maximum times in all the seasons, which was unique in its occurrences. Based on the species distribution apart from *Penicillium*, *Aspergillus* comprised of 10 species, via., *A. awamori, A. flavipes, A flavus, A. fumigatus, A. terreus, A. utuss* and *A. wentii* were recorded in the hospital environment were in agreement with the findings of many others (Dharmage et al., 2002); Hedayati 2005). A number of studies on aerospora particularly of occupational sites are found worldwide (Lacey 1991, 2004). Indoor and outdoor mycospora had a greater resemblance with outdoor qualitatively, but the concentration greatly dependant on atmospheric parameters, both changes with accordance to occurrences, seasonally and diurnally. The present findings found the dense spores in different sites of hospital contribute to the prevailing atmospheric conditions and to the degree of cleanness and activities. The commonly occurrence species in all the season were allowed to grow in SDB media for extracting its bioactive compounds. The bioactive compounds were treated by disc diffusion method for checking the antimicrobial activity of the *Penicillium* spp of different season against bacterial pathogens. Comparative analysis of antimicrobial activity of bioactive compounds of *Penicillium* spp sampled during rainy, summer and winter seasons is given in Fig 1, 2 & 3 respectively.

The zone of inhibition was measured and compared with the control in its raw form and with the different antibiotic disc to confirm their cidal ability. It was found that *E. coli* showed a good antimicrobial activity followed by *S. aureus* in all the seasons where as the least response was from *S. epidermidis and P. vulgaris*. Comparative analysis of antimicrobial activity of *Penicillium chrysogenum, P. citrinum and P. oxalicum* with antibiotics against various pathogens are given in Table 1, 2 & 3 respectively. It was found that the antibiotic effect of bioactive compounds were more in winter months in comparison to other months.

Fig.1 Comparative analysis of antimicrobial activity of bioactive compounds of *Penicillium* spp sampled during rainy season

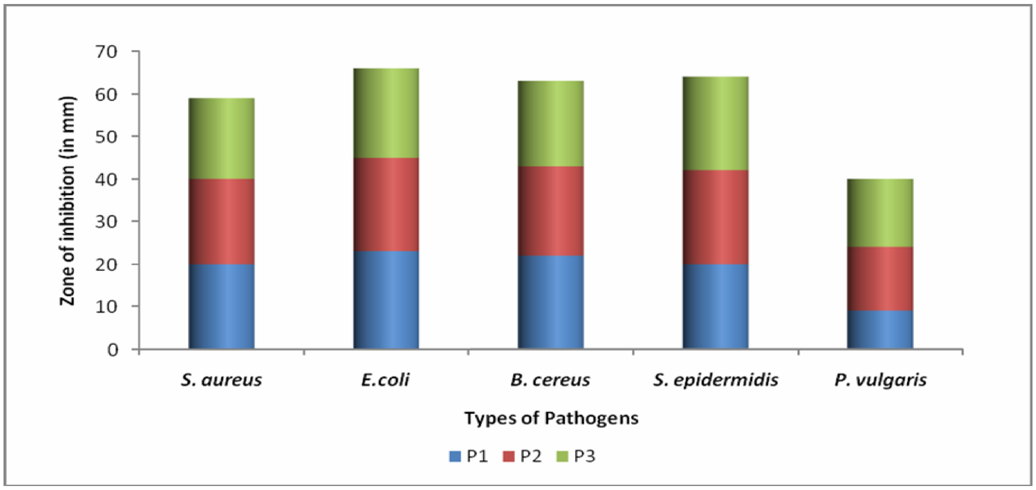


Fig.2 Comparative analysis of antimicrobial activity of bioactive Compounds of *Penicillium* spp sampled during summer season

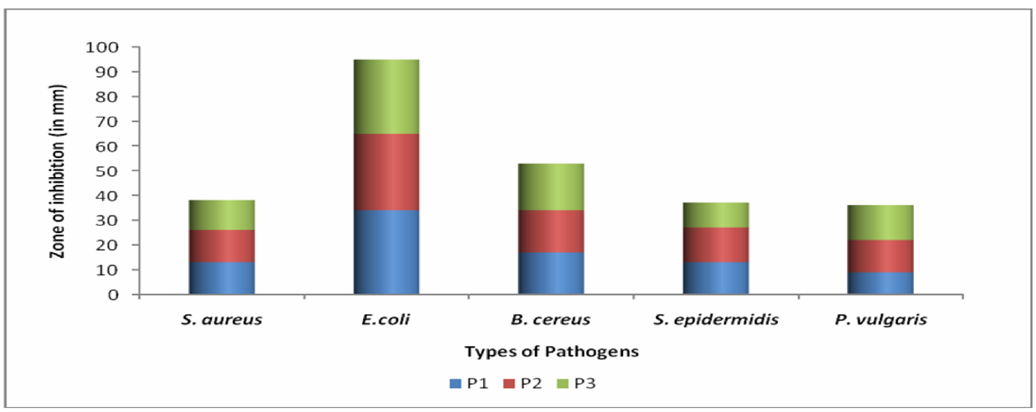


Fig.3 Comparative analysis of antimicrobial activity of bioactive compounds of *Penicillium* spp sampled during winter season

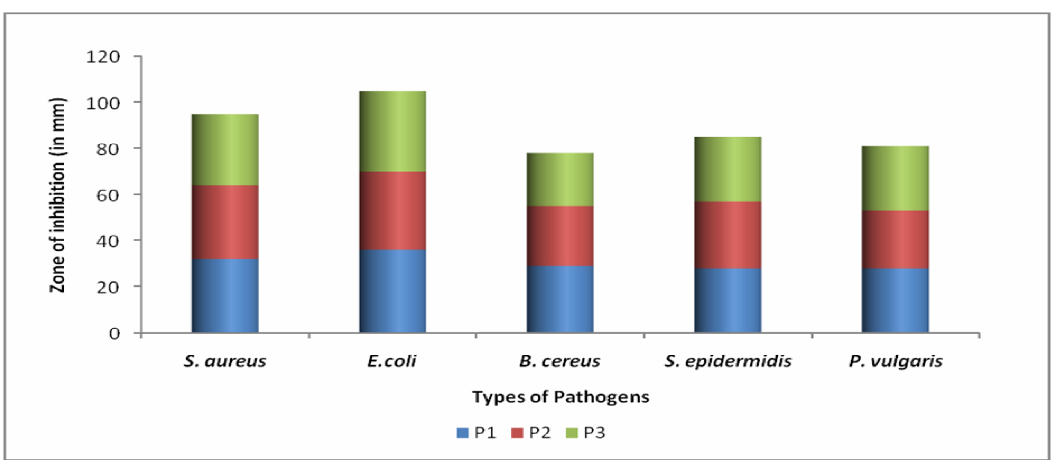


Table.1 Comparative analysis of antimicrobial activity of *Penicillium chrysogenum* with antibiotics against various pathogens

Pathogens/ antibiotics	Tetracycline (30mcg) ZoI in (mm)	Vancomycin (30mcg) ZoI in (mm)	Ampicillin (10mcg) ZoI in (mm)	Amoxicillin (10mcg) ZoI in (mm)	Penicillin-G (10mcg) ZoI in (mm)
S. aureus	**31**	**18**	**11**	**14**	**8**
E. coli	**24**	**7**	**9**	**9**	**10**
B. cereus	**29**	**20**	**14**	**16**	**8**
S. epidermidis	**28**	**22**	**14**	**18**	**9**
P. vulgaris	**29**	**19**	**12**	**15**	**10**

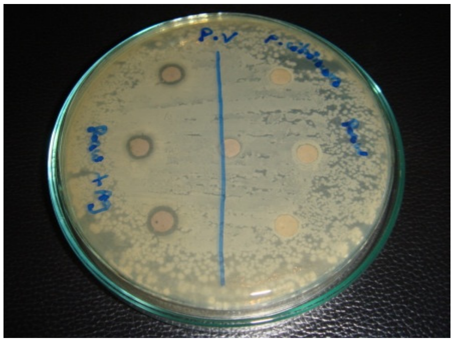
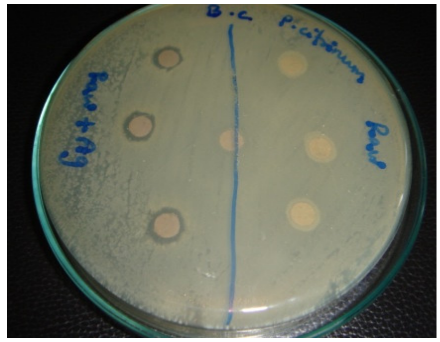
Table.2 Comparative analysis of antimicrobial activity of *Penicillium citrinum* with antibiotics against various pathogens

Pathogens/ antibiotics	Tetracycline (30mcg) ZoI in (mm)	Vancomycin (30mcg) ZoI in (mm)	Ampicillin (10mcg) ZoI in (mm)	Amoxicillin (10mcg) ZoI in (mm)	Penicillin-G (10mcg) ZoI in (mm)
S. aureus	**29**	**19**	**11**	**13**	**9**
E. coli	**22**	**10**	**10**	**11**	**7**
B. cereus	**26**	**21**	**12**	**12**	**8**
S. epidermidis	**24**	**20**	**11**	**11**	**10**
P. vulgaris	**27**	**21**	**10**	**11**	**9**

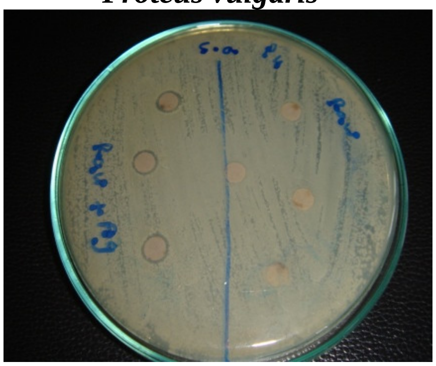
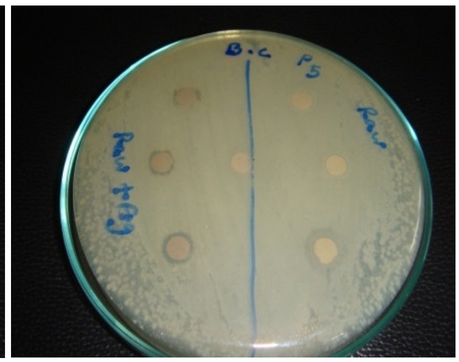
Table.3 Comparative analysis of antimicrobial activity of *Penicillium oxalicum* with antibiotics against various pathogens

Pathogens/ antibiotics	Tetracycline (30mcg) ZoI in (mm)	Vancomycin (30mcg) ZoI in (mm)	Ampicillin (10mcg) ZoI in (mm)	Amoxicillin (10mcg) ZoI in (mm)	Penicillin-G (10mcg) ZoI in (mm)
S. aureus	**30**	**19**	**11**	**12**	**9**
E. coli	**27**	**18**	**19**	**12**	**10**
B. cereus	**27**	**20**	**11**	**11**	**10**
S. epidermidis	**24**	**20**	**8**	**11**	**6**
P. vulgaris	**26**	**17**	**14**	**10**	**9**

Fig.4 Antimicrobial activity of bioactive compounds compared with raw culture of *Penicillium* spp

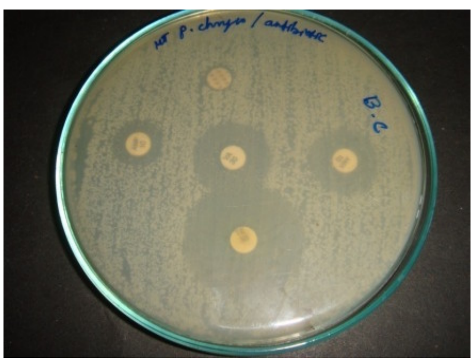



Proteus vulgaris ***Bacillus cereus***

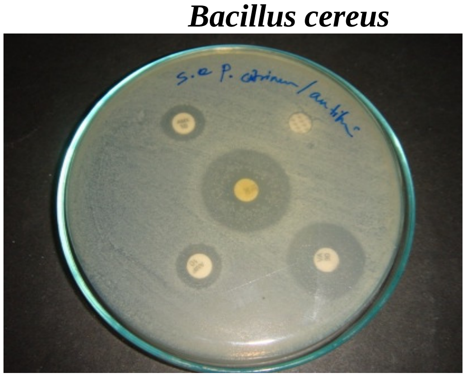
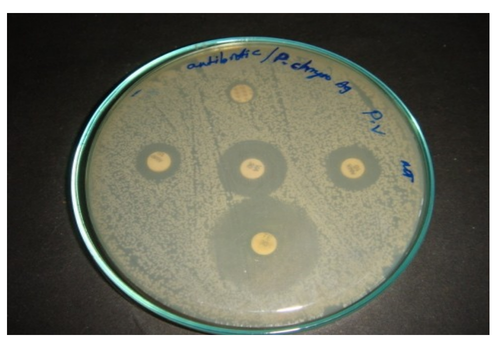



S. aureus ***Proteus vulgaris***

Fig.5 Antimicrobial activity of different antibiotics with bioactive compounds against various bacterial pathogens

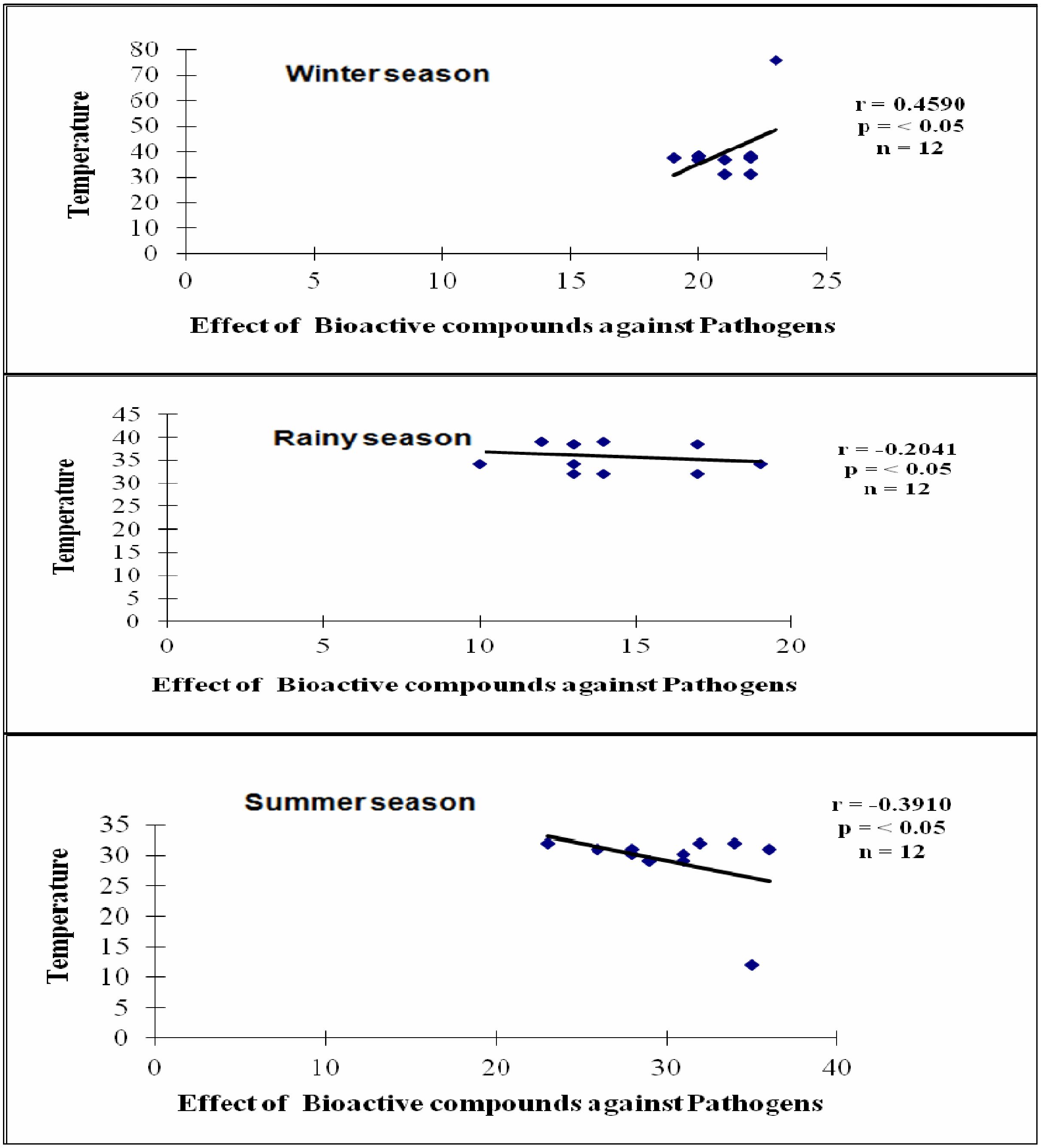



Bacillus cereus ***S. aureus***

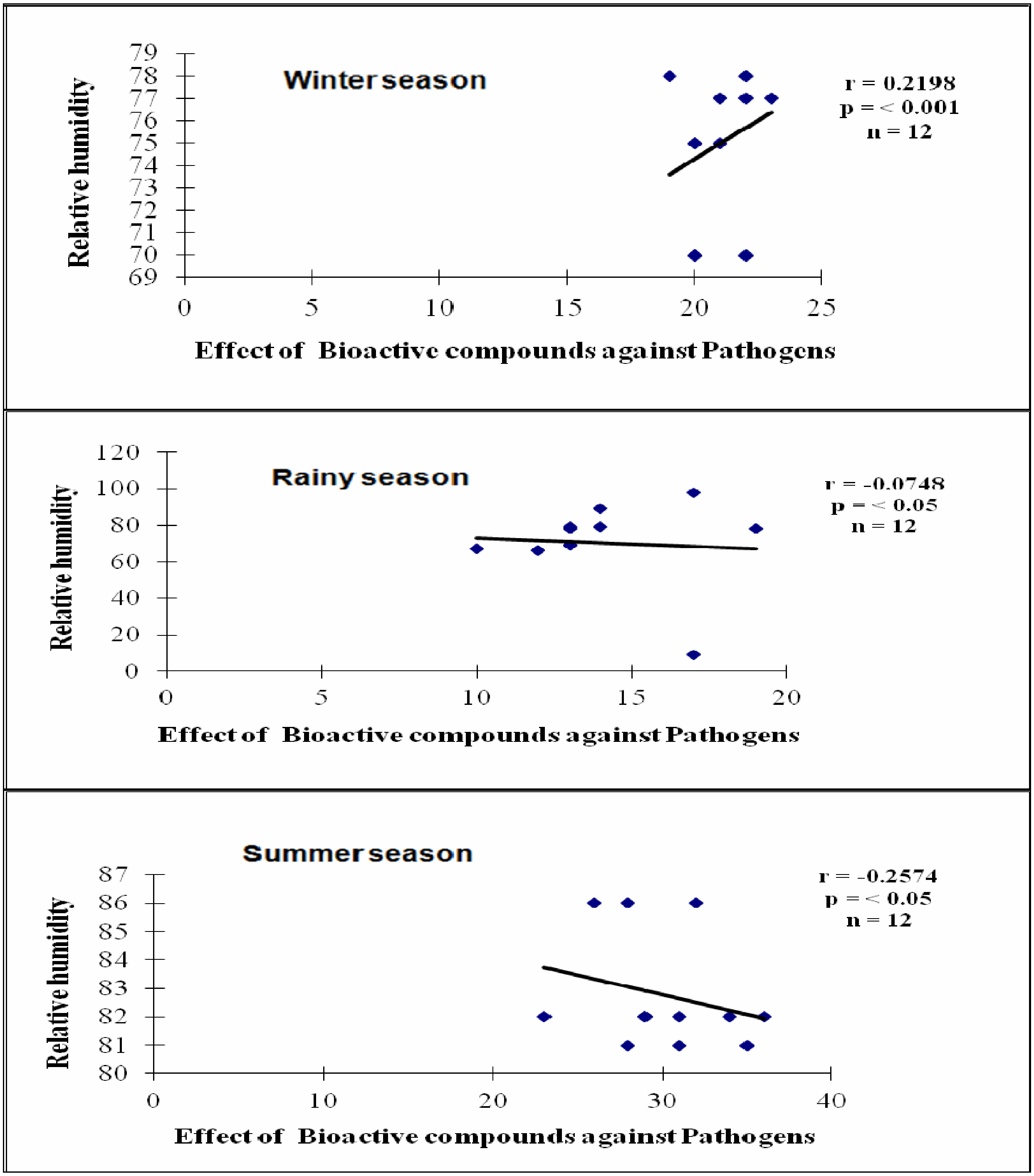
S. epidermidis ***Proteus vulgaris***

Fig.6 Pearson's co-efficient of correlation of seasonal Temperature (^{o}C) with the effectiveness of bioactive compounds from *Penicillium* spp against bacterial pathogens.



'n ' number of observations, 'r' correlation co-efficient and 'p' Probability level

Fig.7 Pearson's co-efficient of correlation of seasonal Relative humidity (%) with the effectiveness of bioactive compounds from *Penicillium* spp against bacterial pathogens**.**



'n ' number of observations, 'r' correlation co-efficient and 'p' Probability level

This study correlated with the report of other researchers that the total number of resistant bacteria was decreased with the increase of distance of sample collection site, the antibiotic susceptibility test of the isolates are multi-drug resistant (≥4) and were resistant to Tetracycline (100%), Ciprofloxacin (100%), Penicillin (100%), Erythromycin (100%), Gentamycin (50%) and Chloramphenicol (90%). Turbidometric analysis of ciprofloxacin resistance pattern showed all of the isolates were highly resistant to ciprofloxacin even at concentration of 3000μg/ml (Islam et al., 2008). Antimicrobial activity of bioactive compounds compared with raw culture of *Penicillium* spp is given in Fig 4 and antimicrobial activity of different antibiotics with bioactive compounds against various bacterial pathogens is given in Fig 5. It was found that the meteorological parameters had direct effect on air borne fungal spores as well as in their activity (Fig 6 & 7). The temperature and relative humidity were found to control the fungal growth and their bioactive production at different seasons of the year.

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