Think India Journal
ISSN: 0971-1260 Vol-22, Special Issue-31
National Conference ETDAB-2019
Held on 23th & 24th December 2019
Organized by: Deptt. of Botany, Deogiri College, Aurangabad, M.S

EFFECT OF DIFFERENT ENVIRONMENTAL CONDITIONS ON THE ANTIMICROBIAL ACTIVITY OF PSEUDOMONAS FLUORESCENS AGAINST ALTERNARIA ALTERNATA(FR.) KEISSLER CAUSING FRUIT ROT OF POMEGRANATE

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ABSTRACT

Fruit rot disease of Pomegranate (*Punica granatum* L). is caused by *Alternaria alternata.* During present investigation Aa-21 resistant isolate of this pathogen was used. Present investigation was undertaken to find out the antimicrobial potential of *Pseudomonas fluorescensin vitro*. Four environmental conditions were testes with different concentration and ranges , exhibited significant mycelial growth inhibition of *A. alternata.* Physical factors such as temperature and P^H influence the activity of biocontrol agent , At 25^0C Percent inhibition was (84.42 %) , at P^H 5.0 (84.77%) , at 30gm carbon source percent inhibition was (82.85%), and at 3gm nitrogen source it was (80.18%). Decrease in concentration of carbon and nitrogen source there is decrease in growth of resistant isolate; carbon and nitrogen have been established as essential elements in the infection process of plant pathogens.

Keywords : *Alternaria alternata,* Fruit rot, *Pseudomonas fluoresence*, Pomegranate, Resistant isolate, Percent inhibition, environmental conditions.

Introduction:

Pomegranate (*Punica granatum* L.) is a member of family Punicaceae. This fruit crop is known as to have been cultivated in the Middle East more than 5000 years ago. Pomegranate is grown as commercial crop in India, Iran, Spain, Morocco, Egypt, Afghanistan, Baluchistan, Burma, China, Japan, and USA. The pomegranate fruit is attacked by many fungal pathogens (Padule and Kaulgud, 1993). Among the fungal pathogens *Alternaria alternata* was very common(Bharade and Kamble, 2001)Twenty one isolate were obtained from fruit rot disease of pomegranate caused by*Alternaria alternata* (Fr.) Keissler. Aa-21 resistant isolatewas used for this sudy. *Pseudomonas fluorescens* is the most commonly used biological control agent and

have long been known as effective antagonists against plant pathogenic fungi (Prasad and Chaudhary 1997). Thus the present study was aimed to evaluate the antagonistic activity of*Pseudomonas fluorescens* in laboratory conditions.

Material and Methods :

Biocontrol of resistant strains of *Alternaria alternata* was studied by using *Pseudomonas fluorescens.* Antagonism of *Pseudomonas fluorescens* against *Alternaria alternata* was tested *in vitro*by streaking the bacteria in 4cm line on one side of the plate in each test medium. Mycelial discs of Aa-21 with 6 mm in diameter) were placed at the other end from the *Pseudomonas fluorescens* on the plate.(Rangashwaran and Prasad, 2000). Each treatment was replicated thrice. Plates were incubated at 26^{o}C and zones of inhibition were recorded after 8 days. Inhibition zone (in mm) was calculated by taking the difference of pathogen growth in control plate and growth in dual culture. To study the effect of varying temperature on the antimicrobial activity of *Pseudomonas fluorescens*, on dual culture plates were incubated at different temperatures i.e. 10^{o}C, 25^{o}C and 35^{o}C. Effect of pH was studied by adjusting different levels of pH in the medium using dilute hydrochloric acid or sodium hydroxide solution. PH ranges were 3.5, 5.0, 6.5 and 8.0. Effect of varying C, N was studied by altering either the amount of carbon source in Czapek Dox medium, variation in only on source was made at a time keeping the quantity of the other constant. The growth inhibition was calculated by using the formula: 100 X C - T / C, Where C = growth in control and T = growth in treatment (Vincent, 1947). Statistical analyses of the experiments were performed by using the book of Mungikar (1997).

Result and Discussion:

Data presented in Table 1 indicated that *Pseudomonas fluorescens* showed antimicrobial activity. Several workers have been reported that the use of *Pseudomonas fluorescens* against number of plant pathogenic fungi (Baker and Cook (1974) , Howell and Stipanovic (1979), Vidhyasekaran and Muthamilan (1995), Bhattacharya and Pramanik (1998), Manoranjitham *et al.* (2000) reported that soil application of *T.viridae* and *Pseudomonas fluorescens* effectively checked the pre-emergence and post emergence damping off of tomato caused by *Pythium aphanidermatum.*

Conclusion:

In conclusion, *Pseudomonas fluorescens* evaluated *in vitro* was found potential antimicrobial agent against *A. alternata.* with significantly highest inhibition of mycelial growth of the *A. alternata* causing fruit rotof pomegranate. Considerable crop losses incurred by plant diseases caused by phytopathogenic agents. Interest in biological control of fungal pathogens

has increased recently in order to find alternatives to the use of chemicals. Synthetic pesticides are costly, pollute the environment and are potentially harmful to animals and humans. Furthermore, their repeated use promotes the development of chemically resistant pathogen strains. The use of microbes for pest management in agriculture is one of the most effective strategies of biological control.

Table 1.*In vitro* evaluation of antimicrobial agent against mycelial growth of*A. alternate*indual culture.

Sources	**Control growth *Diameter without *Pseudomonas fluorescens* (mm)**	**Growth *Diameter with *Pseudomonas fluorescens* (mm)**	**Percent (%) Inhibition**
Carbon			
30 gm	90.00	18.00	82.85
15gm	60.00	12.66	81.18
7.5gm	55.00	10.00	80.00
Nitrogen			
3gm	90.00	18.66	80.00
1.5gm	74.00	14.66	80.18
0.75gm	65.00	12.66	80.00
S.E.±	5.63	3.26	20.00
C.D.(P=0.01)	14.46	5.11	51.00
Temperature			
10^0	28.00	8.00	71.42
25	80.00	11.66	84.42
35	00.00	00.00	00.00
P^H			
3.5	65.00	12.66	80.52
5.0	70.00	10.66	84.77
6.5	89.00	15.66	82.20
8.0	73.00	13.33	81.73
S.E. ±	7.00	0.30	28.00
C.D.(P=0.01)	20.25	2.31	60.00

*Diameter: Mean of three replications.

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