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Optimization & Molecular Characterization of an Antifungal Metabolite (2,4-diacetylphloroglucinol) from *Pseudomonas fluorescence in Management of Fusarium wilt in soybean*

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Abstract: The bacteria *Pseudomonas* is being used as biological control agent and it is safe alternative for the fungicide. Our objective was to optimize the nutrition and environmental condition for biomass and antifungal efficiency and to evaluate it against wilt disease caused by F. *Oxysporum* in soybean. *Pseudomonas fluorescence,* showed antagonistic properties, in vitro, against the pathogen *Fusarium oxysporum* by dual culture. Effect of the separated secondary metabolites on the fungal growth by broth dilution technique and antifungal activity by agar well diffusion technique was studied. The present studies, purified metabolite of the *Pseudomonas fluorescence* inhibited the *Fusarium oxysporum* at the concentration of 0.5% that was analyzed by well diffusion method. The secondary metabolites was subjected for TLC. Further, purified effective metabolite was analyzed by HPLC and GC-MS with its Retension time molecular weight as 2,4 DAPG. In our studies we optimized, the metabolite concentration by OVAT. The number of variable factors was optimized showed that Glucose, Peptone and mineral salts are significantly increasing the production of DAPG up to 38.47ug/ml. Furthermore, RSM suggested that glucose peptone and mineral salt would result in the maximum production of the 2,4Diacetyl Phloroglucinol 46.5 ug/ml. Under natural condition, *P. fluorescence* formulation was effective in reducing Fusarium wilt in soybean. Seed treatment with *Pseudomonas* protected crop from disease in comparing of fungicide. In addition, the obtained results showed that bacterial treatment significantly increased the growth parameters as well as dry weights and yield.

Keywords: 2,4-Diacetylphloroglucinol, Biocontrol, Metabolite, *Pseudomonas*, Fusarium

1. Introduction

Soybean (Glycine max (L.) Merrill) is vital crop in India and mainly grown in Kharif season. Madhya Pradesh being ranked first in area and production has occupied 54.01 lakh ha area with the typical productivity of 1020 kg/ha and total production is 55.06 lakh ton [1]. Magnificent progress in the soybean productivity is observed within the recent years. This productivity is showing a declining trend due to arising of biotic distress in its cultivation [2]. Soybean is affected by 100 pathogens out of which 66 fungi, 6 bacteria, 8 viruses and 7 nematodes are responsible for devastating infestation [3]. The soybean crop is presently suffering due to one of the disease, which is known as sudden death syndrome, or wilt of soybean [4]. Fusarium wilt caused by *F. oxysporum* is one of the most destructive disease of the crop and it is a very common soil-borne fungus throughout the world. According to the Sinclair and Blackman [5], *F. oxysporum* can reduce the average yield of soybean up to 59%. However, the management of this pathogen is difficult because of its long persistence in soil and wide host range. Some chemical fungicides are effective against this fungus but these chemicals are expensive and harmful for living things as well as the environment [6]. Carbendazim, a systemic fungicide has extensive application worldwide [7] to regulate fungal diseases of plants. Extensive

use of fungicide has resulted in fungicide resistance [8]. The fungicide seed treatment has resulted in decreased plant nodulation, which consequently reduced the grain yield [9, 10].

Looking to this integrated approach could be a possible approach in which the biological control is integrated with other control measures because effectivity of these methods differ according to the time and locations. Most commonly used bio fertilizers and biocontrol agents are grouped together as plant-growth-promoting rhizobacteria (PGPR). The majority of the most well-known PGPR belong to the genera *Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia. Enterobacter, Klebsiella, Pseudomonas, Rhizobium, and Serratia* [11].

Amongst various PGPR, fluorescent *pseudomonads* have been exploited for plant growth promotion and suppression of crop diseases [12, 13]. It has immense potential for suppression of fungal pathogen by producing secondary metabolite [14]. The rhizobacteria are dependent on root exudate for the nutritional requirements specially the carbon and nitrogen source. Before optimizing the metabolite production the nutritional and physiological demands should be studied [15]. The previous studies suggested that changes in physical parameters and media components [16, 17] influenced the antifungal metabolite production. Previously in order to fine tune the precise fermentation conditions the significant components are determined by OFAT (one factor at a time). These significant factors and their interaction to optimize the product output are studied statistically by RSM (response surface methology). In the present study, we optimized media component & physical parameters for characterized antifungal metabolite of *Pseudomonas fluorescence* (DAPG) production by using OVAT and RSM after confirming its inhibitory action against *Fusarium oxysporum*.

2. Materials & Method

2.1. Microorganisms

The selected *Pseudomonas* was procured from Department of Microbiology, Barkatullah University, Bhopal (MP). The selected strain maintained on King's B medium the strains. The test fungal isolate *Fusarium oxysporum* (MTCC-8608) was obtained from the ICAR, Indore. The fungal culture was maintained on PDA medium.

2.2. Extraction of Antifungal Metabolites

The antifungal metabolite is separated from culture supernatant by the method described by S. R. Prabhukarthikeyan [18] with slight modification, in which the culture was grown on king's B medium (50ml) at 30°C in rotatory shaker at 150RPM for 4 days. The culture filtrate was acidified to pH 2.0 with 1N HCL. The acidified filtrate was then extracted 2 times with equal volume of ethyl acetate with the help of the separating funnel. The organic layer separated was evaporated with rotatory evaporator at 45°C. The dried filtrate was then dissolved in methanol and stored at-20°C. The culture filtrate (5ul) was spotted onto silica gel plate (Merck, Silica gel 60 F254, Germany). The plates were developed with Isopropanol: Ammonia: Water (8:1:1) and visualized by short wave length (254 nm). For specific antibiotics the Rf values was calculated. The spots on the TLC plate are scratched and studied for their inhibitory effect. The corresponding spot showing maximum inhibition was again confirmed with the suitable mobile phase.

For the detection of an antibiotic, 2, 4- DAPG, a volume of 5 ul of sample was spotted on to the aluminium coated sheets with silica gel. Separation was performed with acetonitrile/ methanol/water (1:1:1) as a solvent system and visualized by short wave length (245 nm) and diazotized sulphanilic acid. Rf value for the spot confirming 2, 4-DAPG was calculated. The corresponding spot was collected and extracted with 100% acetone. The acetone containing solution was centrifuged and the supernatant was transferred to a fresh appendorf tubes.

The supernatant was analyzed by HPLC as per [19]. In this method filter size, 0.22 micrometer was used. 20 microliter of the sample was loaded with the gradient flow of mobile phase having 1% Triflouro acetic acid and 100% acetonitrile. Flow rate was maintained at the rate of 1ml/min. The peak was observed at 270nm. The purified metabolite collected from HPLC at desired retention time was dissolved in ethyl acetate (5 ml) and filtered to remove insoluble impurities. The Chemstation Agilent software was used for integration of chromatograms, and quantitation of DAPG was done according to a standard curve with a chemical standard (Sigma-Aldrich). This experiment was done three times independently. The ethyl acetate was allowed to evaporate & residues then dissolved in minimum quantity of acetone and analyzed by Mass spectroscopy.

2.3. Growth and Antifungal Activity of Purified Metabolite

For the measurement of growth turbidity was measured from the UV-visible spectrophotometer (schimadzu). The optical density was measured at 600nm.

Antifungal activity of ethyl acetate extract of culture filtrate was performed by well diffusion method [20] with slight modification, in which the Fusarium containing 10^7 conidial spores/ml is spreaded on the plate. The culture filtrate in the different concentration (0, 0.5, 1.0, 1.5, and 2%) was added in the 9 mm well (0.1 ml) incubated at 25°C for 7 days. After incubation, the plate was observed for the zone of inhibition as compare to control in which only solvent was mixed with PDA.

2.4. Selection of the Suitable Medium

King's B medium (KB), nutrient broth (NB), Sucrose Asparagine Medium (SAM), Potato Dextrose Broth (PDB) and Pigment Production Medium (PPM). Each Erlenmeyer flask (250 ml) containing 50 ml of medium was inoculated with 1% inoculum and incubated at 28°C and 150 RPM for 4 days. Cell-free culture filtrate was analyzed for antifungal

activity. After selecting the suitable medium different conditions like Temperature, pH, RPM, Incubation time was also optimized by keeping the suitable medium at different physiological conditions.

2.5. Effect of Variable Carbon Sources

In this experiment, glycerol (10g/l) in the medium was replaced by different carbon sources like Glucose, Fructose, Sucrose, Maltose, Xylose was studied. Erlenmeyer flasks (250 ml) containing 50 ml of the medium devoid of its own carbon source and containing the equivalent amount of other carbon sources were prepared, inoculated and incubated. At the end of the incubation period, samples were removed for determination of optical density and inhibition zone.

2.6. Effect of Variable Concentrations of the Selected Carbon Source

Flasks (250 ml) with 50 ml aliquots of the medium containing different concentrations of the carbon source that showed promising results were prepared. Carbon source (glucose) was added at different concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 gm/50 ml). The flasks were sterilized, inoculated and incubated as described above. At the end of the incubation period, samples were removed for determination of optical density and inhibition zone.

2.7. Effect of Different Nitrogen Sources

This was studied by replacing peptone of medium with other tested nitrogen sources. The tested nitrogen source was added at a concentration equivalent in nitrogen content to that of medium (20gm/lit). The studied nitrogen sources were classified into organic (urea, yeast extract, peptone) and inorganic sources (sodium nitrate, potassium nitrate, ammonium nitrate). Erlenmeyer flasks (250 ml) containing 50 ml aliquots of king's B medium devoid of its own nitrogen source and containing the equivalent amount of other nitrogen sources were prepared, inoculated and incubated as described above. At the end of the incubation period, samples were removed for determination of optical density and zone of inhibition.

2.8. Effect of Variable Concentrations of the Selected Nitrogen Source

Flasks (250 ml) with 50 ml aliquots of the medium containing different concentrations of the promising nitrogen source (0.5, 1.5, 2, 2.5, 3, 3.5, 4 gm/50ml) that showed promising results were prepared. The flasks were sterilized, inoculated and incubated as described above. At the end of the incubation period, samples were removed for determination of optical density and zone of inhibition.

2.9. Effect of Different Mineral Salt

According to the previous studies of effect of mineral amendment on the enhanced DAPG production, the different mineral salt ($ZnSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, H_3BO_3, Na_2MoO_4, $CoCl_2 \cdot 6H_2O$ $NiCl_2 \cdot 6H_20$) was added. 1Mm concentration of each metabolite was added individually in the media. The flasks were sterilized, inoculated and incubated as described above. At the end of the incubation period, samples were removed for determination of optical density and zone of inhibition.

2.10. Optimization of Significant Interacting Component by RSM

The RSM technology and Box Benkhen design was used to optimize the concentration of significant interacting factors that could give the maximum possible production of the desired metabolite. Three significant variables so obtained by OFAT (Glucose, Peptone, and mineral salt) are taken in to consideration. Three levels of the factors are coded as +1high level, intermediate level coded as 0 and the low levels are coded as -1. The coded values are given in Table 1. Total 19 experimental run with triplicate was performed. Design expert 12.0 (Stat –Ease, Inc, Minneapolis, USA) was used for experiment design as well as for the statistical analysis of the data ANOVA and regression analysis of the statistical model was performed. In order to validate the experimental findings validation experiment was carried out under proposed ideal conditions. The purified component was the quantified and its antifungal property was studied.

3. Results

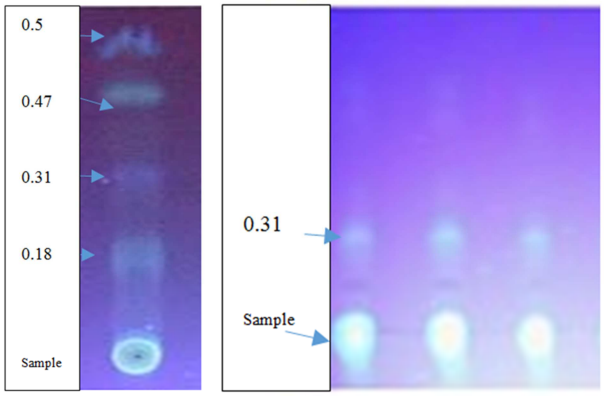

Figure 1(a). TLC of culture fliterate. Figure 1(b). TLC of purified extract.

Figure 1. *Thin Layer Chromatography for crude extract of Pseudomonas fluorescence.*

In order to identify the effective metabolite ethyl acetate extract was recovered from the *Pseudomonas fluorescence* was spotted on TLC plate. Results of TLC indicated that four metabolites designated as A, B, C and D were produced by strain corresponding by their Rf values of 0.18, 0.31, 0.47 and 0.5 (Figure 1a). When the culture filtrate showing the maximum antifungal potential was extracted with the specific reagents then its TLC had shown Rf value of 0.31 (Figure 1b). Analyzing the different percentage of the metabolite it was observed that compound with Rf value of 0.35 has completely inhibited

Fusarium at 0.5% by well diffusion method. HPLC spectrum has shown a peak at 14.635 min and concentration of metabolite detected was found 23ug/ml (Figure 2). Molecular weight was estimated at M/z 210 by mass spectroscopy which agreed with the composition of $C_{10}H_{10}O_5$ for 2, 4 DAPG (Figure 3).

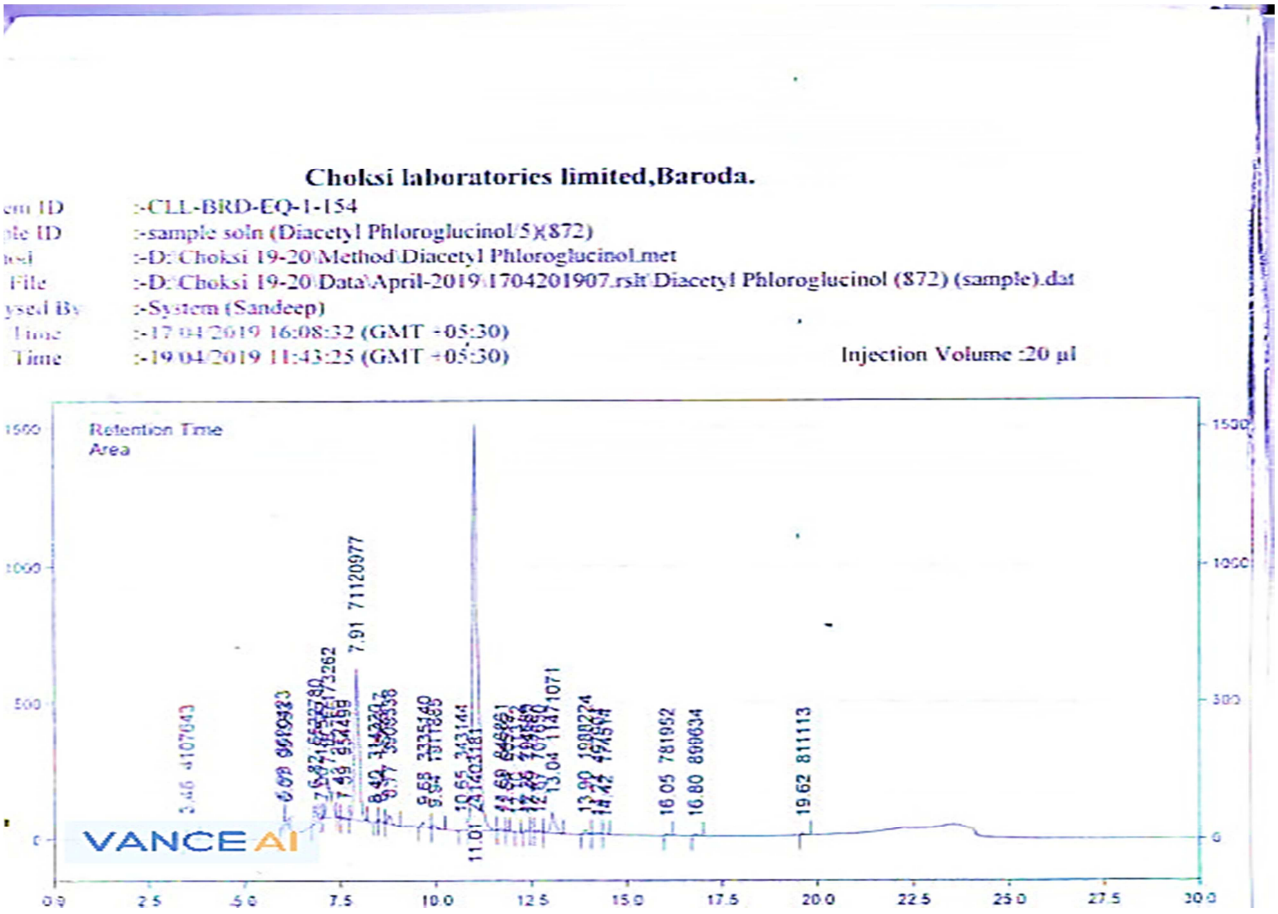

Figure 1. *HPLC analysis of DAPG.*

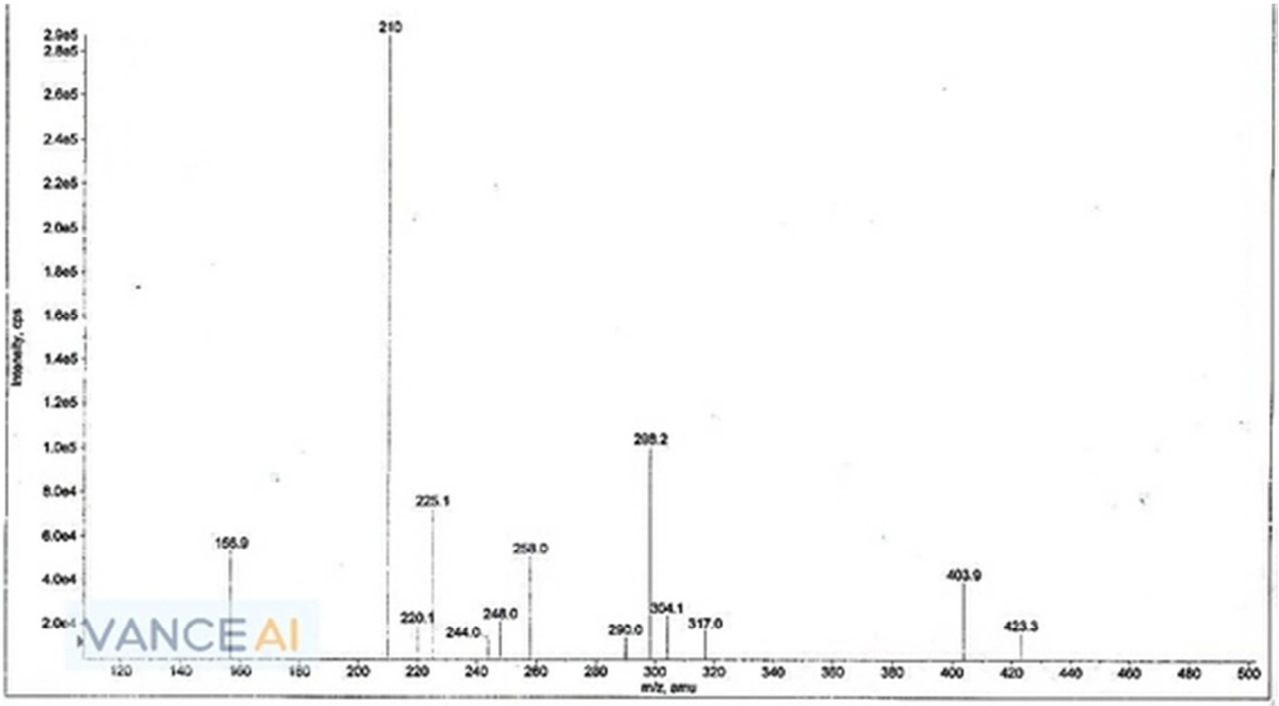

Figure 2. *Mass spectroscopic analysis of DAPG.*

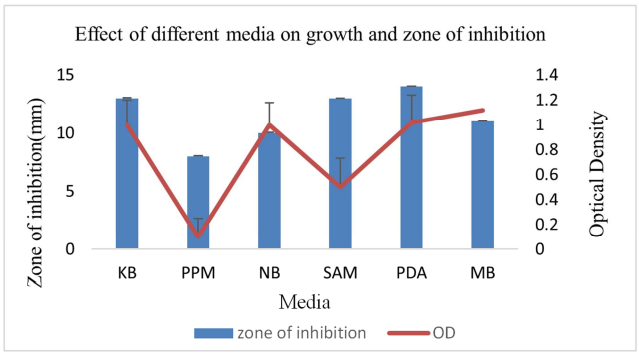

Figure 4. *Effect of different Media on growth and zone of inhibition.*

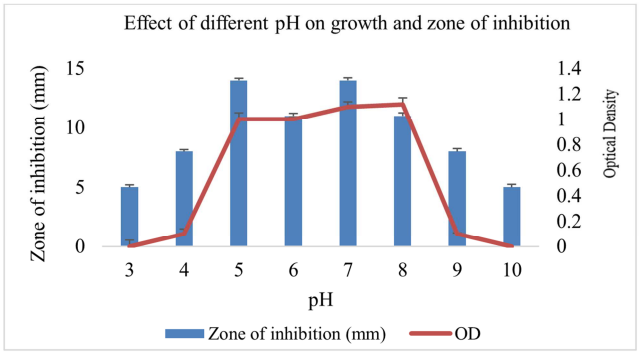

Figure 5. *Effect of different pH on growth and zone of inhibition.*

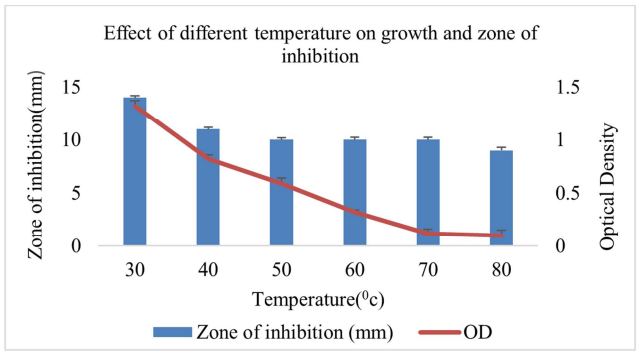

Figure 6. *Effect of different temperatures on growth and zone of inhibition.*

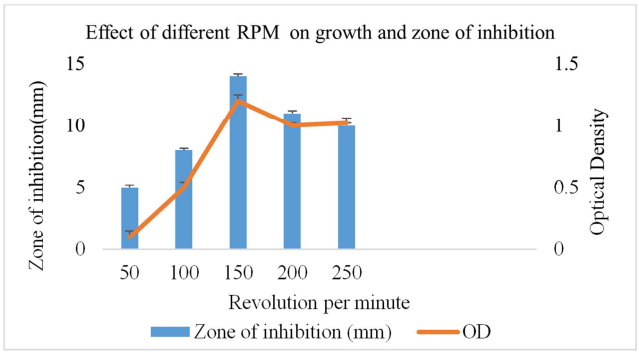

Figure 7. *Effect of RPM on growth and zone of inhibition.*

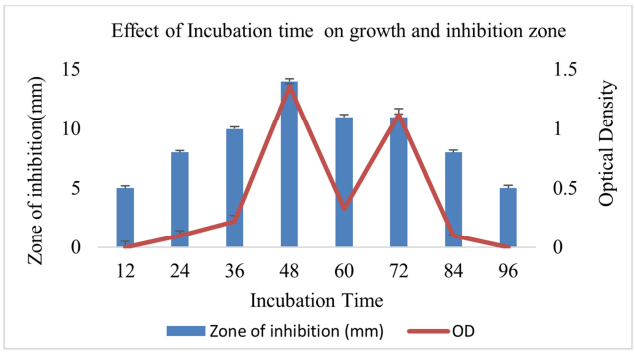

Figure 8. *Effect of Different Incubation time on growth and zone of inhibition.*

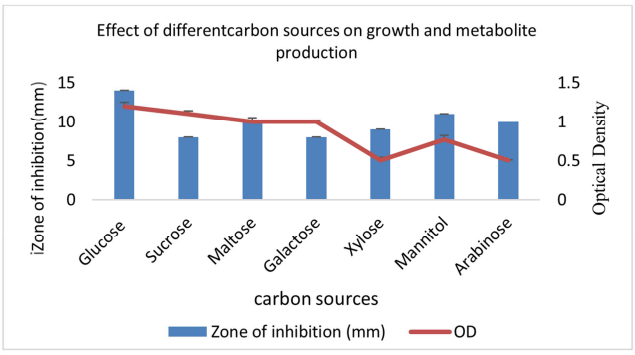

Figure 9. *Effect of Different C-sources on growth and zone of inhibition.*

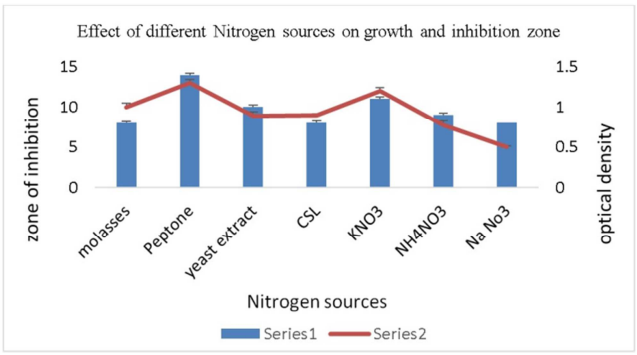

Figure 10. *Effect of different N- sources on growth and zone of inhibition.*

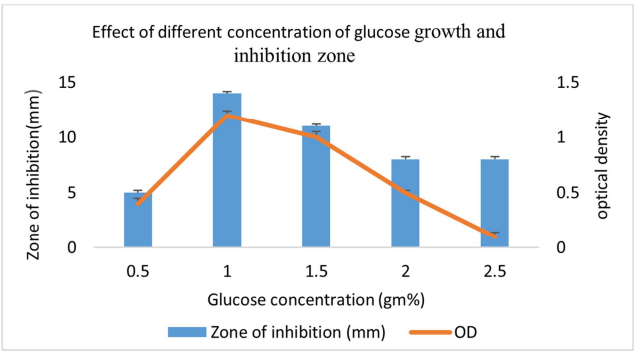

Figure 11. *Effect of different glucose concentration on growth and zone of inhibition.*

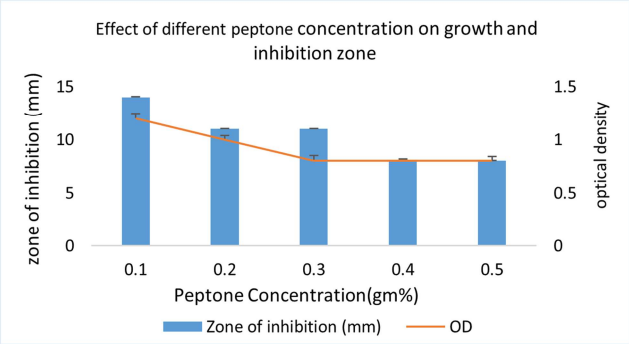

Figure 12. *Effect of different peptone concentration on growth and zone of inhibition.*

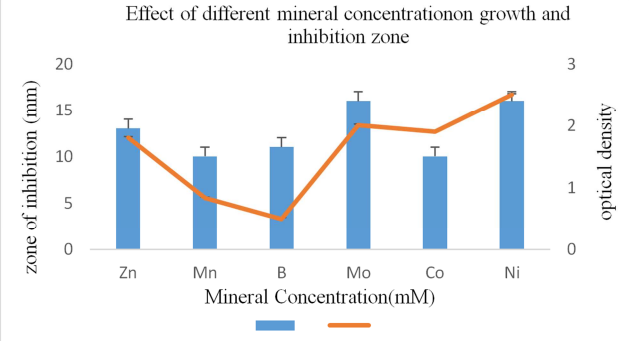

Figure 13. *Effect of Different mineral concentrations on growth and zone of inhibition.*

Different media like Nutrient Broth, Potato Dextrose Agar, Glucose Yeast Extract, Sucrose Aspargine Medium, Pigment Production Medium and King's B medium. Highest optical density and maximum zone of inhibition was observed in King's B medium (Figure 4). The presence of glycerol, phosphate in the king's B medium has favored the production of desired metabolite The King's B medium was then selected for the further optimization of physiological parameters. The maximum zone of inhibition and maximum optical density was observed at pH 5 (Figure 5). The maximum zone of inhibition and maximum optical density was observed at 30°C (Figure 6). The incubation time and RPM was standardized. The maximum zone of inhibition and maximum optical density was observed at 150 RPM and 48 hours, which remained static up to 72 hours and then observed a decrease in antifungal activity. (Figures 7 and 8). Different strains of *Pseudomonas* has shown varied response of DAPG production in different carbon sources. Efficiency of DAPG production was also studied on different nitrogen sources. In the different nitrogen sources, peptone showed optimum antifungal activity (Figures 9 and 10).

In present study, various concentration of carbon and nitrogen sources was also examined to determine the suitable concentration for the maximum DAPG production. Glucose concentration at 1% and peptone concentration at 0.1% showed maximum metabolite activity. (Figures 11 and 12). In this study out of different carbon sources Glucose has favoured the optimum DAPG production. Out of the different mineral salt added only Nickel, molybdenum had shown improved antifungal activity and optical density as compare to other metabolite. The selected factors (Glucose, Peptone and mineral salt) with the optimum physiological conditions was selected as per OVAT for DAPG production. Under the optimized condition with OVAT optical density of 1.5 and maximum zone of inhibition 16.8 mm was obtained.

The purified extract had shown 35.48 ug/ml of DAPG. The OFAT method had shown 16% increase in DAPG production. These factors was further assessed by statistical optimization method using Stat –Ease software. The BBD involves 4 stages of optimization execution of statistical design, prediction of mathematical model on the basis of ANOVA, controlling the efficiency of expected model with diagnostic plots, validation of model by second order quadratic term. The statistical design along with their response are depicted in Table 1. The predicted regression equation that was followed for the given response as the function of coded values was as:

$$Y=+42.71933+3.63583A+1.51404B+2.46594C+1.78750A*B -0.087500A*C+1.03750B*C-3.00556A^2-3.80105B^2- 3.80105C^2$$

Where y is antifungal activity of metabolite measurements zone of inhibition (mm). The correlation between the actual and predicted values obtained from the model are given in (Table 2). The result of ANOVA showed that the variance of observation for two level experiment showed that the difference was significant. The model F- value of 4.59and value of P>F (0.0166) indicated the significance of the model. The regression analysis of the RSM is given in Table 3, which is showing that the independent variable for antifungal activity had shown R^2 value of 97%, which is obtained from dependent variables of glucose, peptone and mineral salt.

Table 1. *BBD analysis with their response.*

Run	A:Glucose	B:peptone	C:mineral salt	Zone of inhibition
	%	%	mM	(MM)
1	-1.68179	0	0	25
2	0	0	1.68179	37
3	0	1.68179	0	37
4	0	0	0	43
5	0	0	0	39
6	1	-1	1	36
7	1	-1	-1	27.5
8	0	0	-1.68179	27.5
9	1	1	-1	36.5
10	0	0	0	45.5
11	0	0	0	43
12	1	1	1	36.5
13	1.68179	0	0	44
14	-1	1	1	35
15	-1	-1	1	29
16	0	-1.68179	0	27.5
17	0	0	0	43
18	-1	-1	-1	32.8
19	-1	1	-1	22

Table 2. *ANOVA analysis of predicted model.*

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	731.61	9	81.29	4.59	0.0166	significant
A-Glucose	180.53	1	180.53	10.18	0.0110	
B-peptone	31.31	1	31.31	1.77	0.2166	
C-mineral salt	83.05	1	83.05	4.68	0.0587	
AB	25.56	1	25.56	1.44	0.2605	
AC	0.0612	1	0.0612	0.0035	0.9544	
BC	8.61	1	8.61	0.4857	0.5034	
A^2	123.31	1	123.31	6.96	0.0270	
B^2	197.22	1	197.22	11.12	0.0087	
C^2	197.22	1	197.22	11.12	0.0087	
Residual	159.55	9	17.73			
Lack of Fit	137.75	5	27.55	5.06	0.0708	not significant
Pure Error	21.80	4	5.45			
Cor Total	891.17	18				

Table 1. *Regression analysis of RSM.*

Std. Dev.	0.9416	R^2	0.9700
Mean	41.68	Adjusted R^2	0.9400
C.V. %	2.26	Predicted R^2	0.7456
Adeq Precision	16.1841		

The optimum factors & their interaction was depicted in 3D graph (Figures 14 and 15). Figure 14 describes the relative effect of glucose and peptone by keeping the mineral salt concentration at its central value of 0.5 Mm. The response variable zone of inhibition is strongly dependent on peptone concentration. Figure 15 depicts the relative effect of glucose and mineral salt by keeping the peptone concentration at its central value of 0.05 %. The glucose and mineral salt concentration inhibit the activity of metabolite at lower concentration and the activity found to be increase at higher concentration. Figure 16 depicts the relative effect of peptone and mineral salt by keeping the glucose concentration at its central value of 0.5 %. The peptone and mineral salt concentration inhibit the activity of metabolite at lower concentration and the activity found to be increase at higher concentration but the enhancement of the activity was observed maximum when the peptone and mineral salt concentration is increased at constant glucose concentration. The validation experiment was performed in large-scale fermenter (2500 ml) under the optimized condition predicted by the model. In this experiment Glucose, Peptone, K_2HPO_4, $MgSO_4.7H_2O$ and mineral salt was added at the concentration of 21.5gm, 1.54gm, 3.75gm, 70 ug with PH 5, RPM 150 for 48 hours. After incubation, the maximum zone of inhibition obtained which was two fold higher than OFAT. The concentration of DAPG was reported 46.7ug/ml, which was 31.6% higher than OFAT.

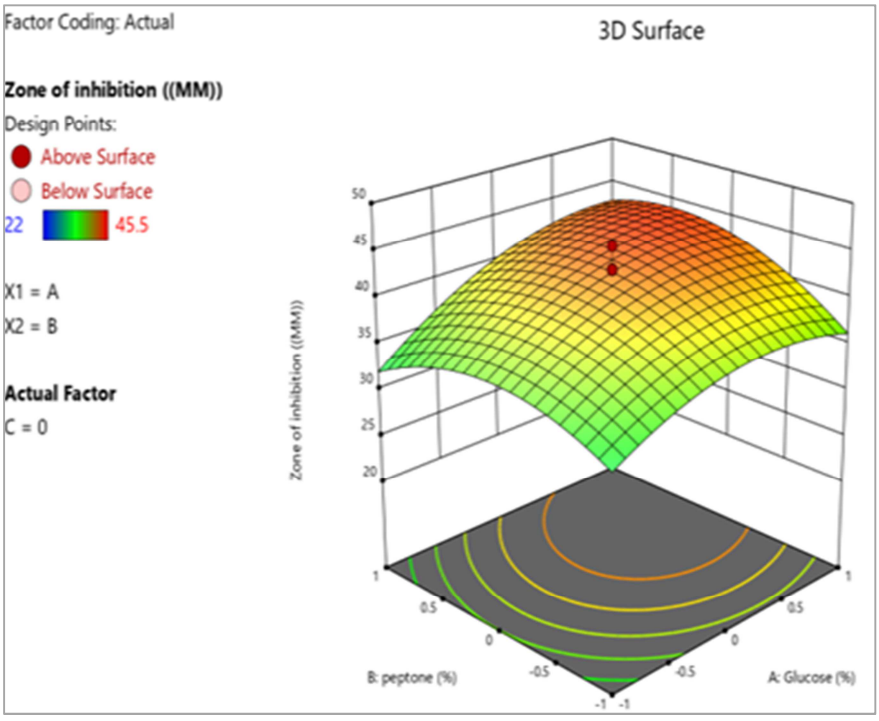

Figure 14. *Three dimensional graph between glucose and peptone on zone of inhibition.*

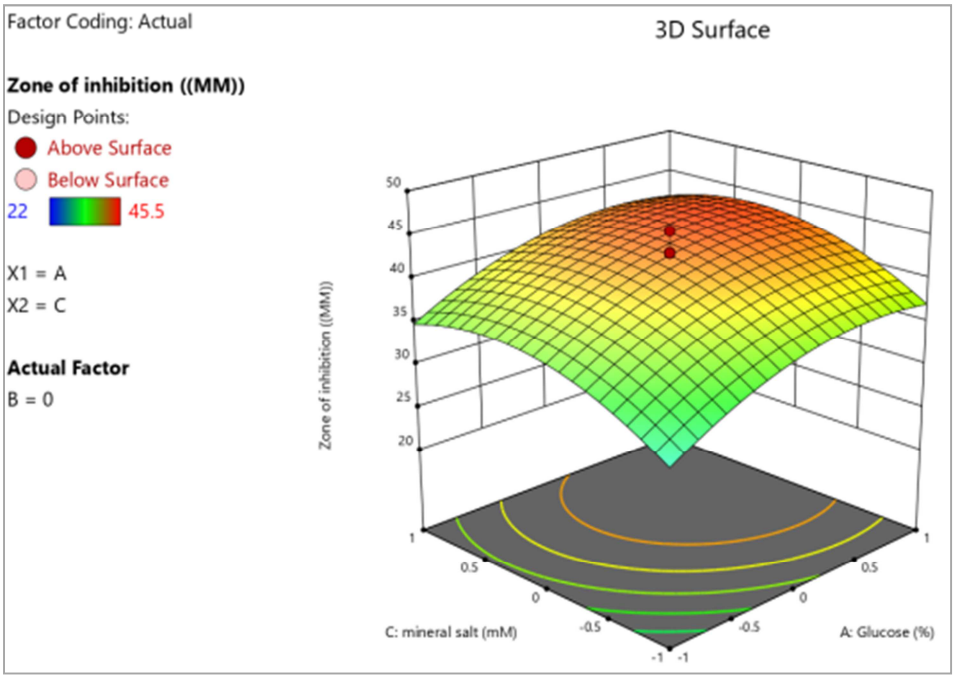

Figure 15. *Three dimensional graph between glucose and Mineral salt.*

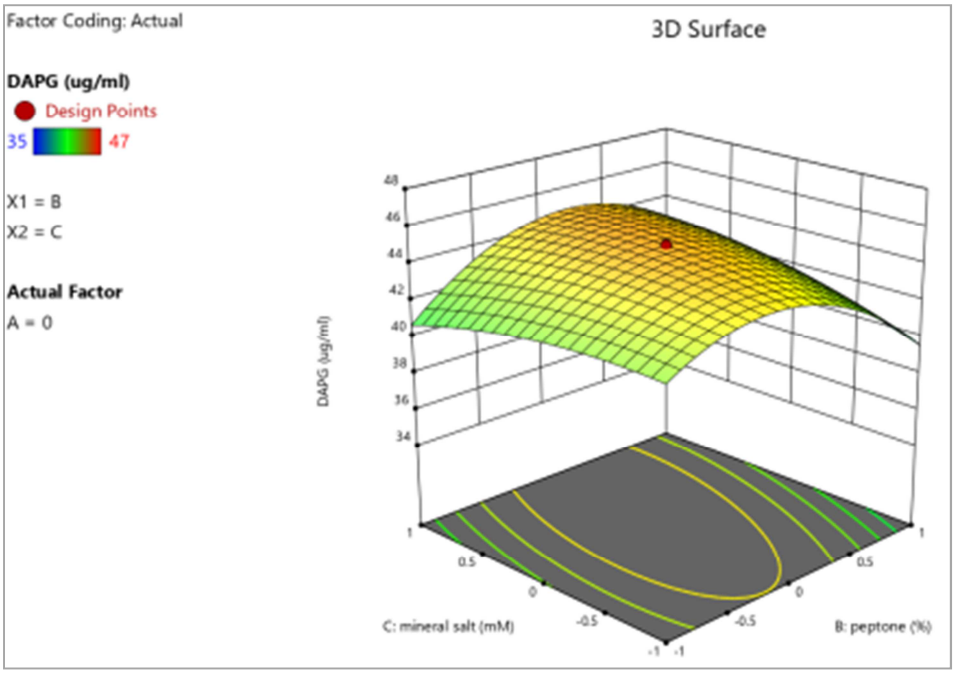

Figure 16. *Three dimensional graph between peptone and Mineral salt.*

Table 4. *Effect of different treatment on Disease incidence and yield.*

Treatment	yield	% increase of yield	Disease incidence	% reduction of disease incidence
Control	5.1 kg/plot		8.2	
Pseudomonas	6.8kg/plot	33%	2	75%
Carbendazim	5.9kg/plot	27%	4	50%

The optimized formulant was added in the pot trial infected with *F. oxysporum* has reduced the disease incidence and increased theyield paremeter shown in Table 4. It is evident from the table that Pseudomonas enhanced the yield by 33% which was 27% in fungicide containning pot.

4. Discussion

In recent days, fluorescent pseudomonads (FPs) have drawn much attention worldwide for the control of fungal

and bacterial pathogens [12, 21, 22] which plays an important role in soil suppressiveness. This microorganism was reported to produce various secondary metabolite viz. Hydrogen cyanide [23], phloroglucinol [24, 25] pyoluteorin [26] and pyrrolnitrin [27]. Fluorescent pseudomonads producing polyketide antibiotic 2,4-diacetylphloroglucinol (DAPG) are an important group of PGPR that suppress root and seedling diseases on a variety of crops [20, 28, 29]. In our study, we also extracted and confirmed the DAPG production from Pseudomonas. This finding corroborate with Reddy KRN [30], who studied that DAPG completely inhibited *R. solani* and the mycelial growth of all test fungi at 0.5 %, observed similar inhibiting concentration. These finding corroborate with Reddy et al. [30] who observed the HPLC peak of DAPG at 11.01 min and molecular weight of 210 for these metabolite.

Significant difference in the biocontrol performance of these bacteria may be observed due to changes in biotic and abiotic factors [31]. Commercial application of this microorganism is hampered due to inconsistent performance in the field. For the successful commercial application of desired metabolite, production should be optimized. Variable quantities of DAPG production is studied [32-34] in natural and flask condition. As per ICAR, bioformulant preparation initial media component (40ml) would be required to prepare 100 gm of the dusting powder based bioformulant. This amount would be diluted 2. 5 times and will not be effective in the field. In order to get the suitable concentration of the effective metabolite media component are selected enhancing the production of DAPG. It has been studied that media composition adversely affect the growth and the accumlation of antimetabolites. The production of 2,4-DAPG in different environmental conditions has been studied both in vitro and in vivo for different strains of Pseudomonas [15, 31, 35]. The physical parameters that will allow the maximum production of DAPG should be selected. To test the effect of temperature various temperature ranges, pH was selected. Similar findings are also reported by Souza et al. [36] in which he observed that DAPG activity against phythium is observed maximum between PH 4.5 -6.5. The lower activity of 2, 4-DAPG at high pH possibly is related to a dissociation of the hydroxyl-substituents of 2,4-DAPG, which may hamper accumulation into the target cell. The similar gene cluster when introduced in the *E. coli* has shown the maximum induction of DAPG between 25-33°C. Temperatures beyond 30°C may reduce enzyme activities related to 2,4- DAPG biosynthesis, which resulted in the lower 2,4-DAPG [37].

The production of metabolite is observed previously also in early log and early stationary phase, which is reported by different researchers [38, 39]. This is confirmed with the pH Profile in which pH was increased sharply up to 8.3, in next 24 hours which remained constant up to 48 hours and then again found to be decreased up to 6.8 in next 72 hours which is stabilized for next 24 hours and found to be decreased up to 6 in next 24 hours. These required RPM supports adequate oxygen supply, which supports the metabolism. The higher agitation speed that could destroy the structure of cell, which will affect the biosynthesis.

Duffy and Defago [15] reported similar findings in which they studied that glucose influenced significantly the production of *Pseudomonas fluorescence.* Kinds of peptone (proteose peptone, peptone & neo peptone) enhanced *B. cepacia, B. multivorans and M. testaceum* on the production of antimicrobial. The peptone itself provides isotonic environment for microorganisms [40]. The changes of nutrients and their concentrations have different effects on the accumulation of different metabolites, which are controlled by intracellular effectors. *P. fluorescens* Pf- 5 enhanced the production of pyrrolnitrin in the liquid medium of diluted corn meal broth and containing 2 µg/mL of supernatant [41]. Different trace metals are previously reported by Saharan K. [42] to enhance the DAPG production from 118 to 135mg/l. The Response Surface method was used for optimization of metabolite production. The analysis suggested the appropriation of the quadratic model for the different factors. For the better understanding of the interaction between different operational factors, experimental design should be employed. The box benkhen design is used here for generating the quadratic surface and parameter optimization.

Results shown are in accordance with the previous researchers M. V. R. K Sarma [43] reported that by the addition of 0.5% tryptophan has increased the DAPG production up to 70mg/liter. The concentration of DAPG obtained in the present study was compared with other published data. It has been reported that a maximum level of 100 mg/L DAPG was achieved with P. fluorescens strain pf-5 using complex growth medium in 72 h [44]. In another study, 0.63 mg/L DAPG was produced in complex medium containing malt and yeast extracts by P. fluorescens Q2-87 [19].

5. Conclusion

Our results suggest that DAPG production in crop rhizosphere is an important factor contributing to suppressive soil. Abundant production potentiality of 2, 4- DAPG is essential for effective management of diseases caused by plant pathogen. Further according to the need of DAPG quantity *P. fluorescens* was grown in optimized media. The highest DAPG so produced could be used effectively for sustainable agrosystem. In the previous researches the complex SM media was used for the optimization of DAPG. We focused on the production of DAPG by using modified simpler media.

Authors' Contributions (Optional)

KP and AP conceptualized the problem and planned the experiments, carried out work, wrote and edited the manuscript.

Conflicts of Interest

Authors declare no conflict of interest.

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