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Evaluation of media for mass production and fermentation of *Pseudomonas* spp. for integrated management of cereal cyst nematode (*Heterodera avenae*)

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Abstract

In this study investigation of different carbon and nitrogen sources in various types of media compositions was made to optimize the fermentation and mass production of *Pseudomonas* spp. Among all the media compositions, the suitable media containing carbon and nitrogen sources were found as media No.9 (Corn flour 50g/L, Glucose 8g/L, Soybean Cake Powder 20g/L, Wheat bran 20g/L, NaCl 4g/L) followed by No.10 (Corn flour 40g/L, Soybean Cake Powder 30g/L, Yeast Extract 5g/L, Wheat bran 20g/L, NaCl 4g/L, KH_2PO_4 5/L, $MgSO_4$ 1.5g/L), No.08 (Corn flour 17g/L, Glucose 10g/L, Soybean Cake Powder 25g/L, Maltose 10g/L, Yeast Extract 5g/L) and No.05 (Corn flour 5g/L, Yeast Extract 1.5g/L, Fish Peptone 2.5g/L, Soybean Cake Powder 20g/L, Peptone 0.75g/L, KH_2PO_4 3g/L, $CaCO_3$ 0.5g/L, $MgSO_4$ 0.3g/L) for the mass production of *Pseudomonas* spp., after 100 hours (4days). The bacterial culture filtrates produced during fermentation in different media compositions showed varied results on the bioassay against the second stage juvenile mortality (J_2) of *H. avenae*. The culture filtrates formed in media No.8 (Corn flour 17g/L, Glucose 10g/L, Soybean Cake Powder 25g/L, Maltose 10g/L, Yeast Extract 5g/L) and media No.5 (Corn flour 5g/L, Yeast Extract 1.5g/L, Fish Peptone 2.5g/L, Soybean Cake Powder 20g/L, Peptone 0.75g/L, KH_2PO_4 3g/L, $CaCO_3$ 0.5g/L, $MgSO_4$ 0.3g/L) has more toxic effects on the second stage juvenile (J_2) mortality as compared to rest of secondary metabolites produced in different media and control treatments. All tested strains of *Pseudomonas* spp. showed stability on pH ranges from acidic pH5 to neutral pH7 and towards basic pH8 against the second stage juvenile (J_2) mortality assay.

Keywords: Media compositions, Fermentation, Secondary metabolites, pH, Cereal cyst nematode *Heterodera avenae*, *Pseudomonas* spp.

Among the genus *Heterodera,* the soil-born cereal cyst nematode (CCN) infects the roots of ancestors and relatives of plants family Poaceae and Gramineae. It has been causing severe economic threshold losses and substantial damage to cereal growing agro-ecologies around the globe (Handoo, 2002; Nicol *et al*., 2003; Dababat *et al*., 2014). The two major species *H. avenae* and *H. filipjevi* are globally recognized infectious to cereal crops (Rivoal & Cook, 1993; McDonald & Nicol, 2005). These species were also reported from China (Chen *et al*., 1991; Li *et al*., 2010; Fu *et al*., 2011). Crop rotation, genetic resistance and chemicals have produced efficient results in the cereal cyst nematode management. Alternative to hazardous chemicals, some other approaches are being explored and considered as environmentally friendly to soil ecology of agriculture production system (Bontempo *et al*., 2014; Riley & Qi, 2015). It includes the development of microbial antagonists and their secreted secondary enzymatic metabolites for integrated management of nematode (IMN) CCN (Riley *et al*., 2010).

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A number of different microorganisms as potential biocontrol agents were evaluated for *H. avenae* and *Pratylenchus* spp. These microorganisms include; *Paecilomyces lilacinus*, *Verticillium chlamydosporium* and *Trichoderma longibrachiatum*, which have been reported having fatal effects on CCN (Siddiqui & Mahmood, 1996, 1999). Bacterial strains have also been used as biocontrol organisms for soil-borne pathogens including insect pests and nematodes (Khan *et al*., 2006; Zhang *et al*., 2014). The bacterium *Pastueria* spp. found pathogenic to insect pests also parasitized *H. avenae.* This bacterium caused 38% to 56% prevention of juveniles to enter into roots (Davies *et al*., 1990). Significant mortality of *H. avenae* juveniles was caused by *Bacillus subtilis*, while effect of *Azobacterium chroococcum* showed 50% reduction in the development of cyst juveniles. The noteworthy effect was observed by actinomycetes isolates from *Streptomyces* spp., which were tested against the motility of *H. filipjevi* second stage juveniles (Bansal *et al*., 1999; Yavuzaslanoglu *et al*., 2001).

Use of bacterial strains as an alternative method for the bio-control of plant parasitic nematodes has been given great attention by nematologists as these methods have dominating influence over the targeted nematodes without any environmental pollution. In soil ecological niche or in suppressive soils, the nematophagous bacteria play an important role in parasitizing; production of toxins, antibiotics or enzymes and nutrients competence; inducing systemic resistance of plants as well as plant health progress (Tian *et al*., 2007).

The bacterium *Pseudomonas* spp., viz., *P. fluorescens* and *P. putida*, the rhizosphere bacteria are highly efficient in reducing soil-born root diseases, insect pests, nematodes in suppressive soils and in turn help in plant growth. The *Pseudomonas* isolates also prevent egg hatching and penetration of juveniles of root-knot nematode *Meloidogyne incognita* in vegetables under protected agriculture, organic farming or tunnel cropping system. Similar effects were observed in *H. avenae* by *Bacillus* spp., strains (Siddiqui *et al*., 2009). A gram negative bacterium *P. fluorescens* CHAO mutant showed reduction in bio-control activity against *M. incognita* under green house (Siddiqui *et al*., 2005).

The objectives of the present research were to investigate the environmental conditions of the indigenous *Pseudomonas* isolates (*P. fluorescens* & *P. putida*) for biomass production. Moreover, the effect of bacterial culture filtrates of these indigenous isolates against the second stage juvenile of cereal cyst nematode *H. avenae* in terms of mortality *in vitro* was studied. The stability of culture filtrates on different pH, on different temperatures and their effect on second stage juvenile mortality *in vitro* were also studied.

Materials and Methods

Bacterial strains: Among the 400 potential bacterial isolates, five selected bacterial isolates of *Psuedomonas* spp.,were; *P. fluorescens* Ps104, *P. fluorescens* Ps109, *P. putida* Ps190, *P. putida* Ps196 and *P. putida* Ps197. These were isolated from inside the cysts of *H. avenae* and wheat roots from susceptible wheat cultivar "Aikang 58 from Agriculture Research Area, Shang Zhuang, China Agriculture University, Beijing. The bacterial isolates were isolated by the culture-dependent method, initially identified by Gram's staining. Both *Pseudomonas* isolates **(***P. putida* and *P. fluorescens*) were cultured on King's medium (King *et al*., 1954). All bacterial strains were preserved in 50% glycerine and kept at -80°C temperature.

Propagation of the selected bacterial isolates in shake flasks: Conical flasks (250 ml capacity) containing 100 ml of Lysogenic Broth (L.B) medium were inoculated by one ml of the selected bacterial isolates then incubated on rotatory shaker GFL (Gesell schaftfür Labortechnik) Laboratory equipment (120rpm) at 28°C for 24h and 48h simultaneously. Different carbon and nitrogen sources were tested to study their effect on bacterial isolates growth (Table 1). Also mortality assay against *H. avenae* juveniles (J_2) and their population reduction (%) were examined *in vitro* conditions.

Table 1. Different media compositions for mass culturing of selected bio-control agents.

Culture Media	Media Composition
Media No.1	Glucose 1g/L, Peptone 10g/L, Yeast Extract 5g/L, $MgSO_4$ 0.2g/L, K_2HPO_4 0.02g/L
Media No.2	Corn flour 40g/L, Soybean Cake Powder 3g/L, Fish Peptone 3g/L, Citric Acid 0.05g/L, KH_2PO_4 5g/L, $CaCO_3$ 3g/L, $MgSO_4$ 2.5g/L, $FeSO_4$ 0.25g/L
Media No.3	Soluble starch 40g/L, Peptone 1g/L, Glucose 20g/L, $NaNO_3$10g/L, KH_2PO_4 3g/L, $MgSO_4$ 1g/L, $CaCO_3$ 0.9g/L
Media No.4	Soya bean cake powder 30g/L, Wheat bran 70 g/L
Media No.5	Corn flour 5g/L, Yeast Extract 1.5g/L, Fish Peptone 2.5g/L, Soybean Cake Powder 20g/L, Peptone 0.75g/L, KH_2PO_4 3g/L, $CaCO_3$ 0.5g/L, $MgSO_4$ 0.3g/L,
Media No.6	Soluble starch 1g/L, Soybean Cake Powder 30g/L, Glucose 15g/L, KH_2PO_4 1.5g/L, $MgSO_4$ 0.5g/L, $MnSO_4$ 1g/L, $CaCO_3$ 0.1g/L, $FeCl_3$ 0.3g/L
Media No.7	Corn flour 5g/L, Glucose 3g/L, Soybean Cake Powder 5g/L, Fish Peptone 3g/L
Media No.8	Corn flour 17g/L, Glucose 10g/L, Soybean Cake Powder 25g/L, Maltose 10g/L, Yeast Extract 5g/L
Media No.9	Corn flour 50g/L, Glucose 8g/L, Soybean Cake Powder 20g/L, Wheat bran 20g/L, NaCl 4g/L
Media No.10	Corn flour 40g/L, Soybean Cake Powder 30g/L, Yeast Extract 5g/L, Wheat bran 20g/L, NaCl 4g/L, KH_2PO_4 5/L, $MgSO_4$ 1.5g/L,
Media No.11	Corn flour 10g/L, Glucose 5g/L, Soybean Cake Powder 15g/L, Fish Peptone 5g/L, $CaCO_3$ 5g/L , $MgSO_4$ 0.2g/L, $MnSO_4$ 0.2g/L, Ammonium Sulphate 1g/L
Media No.12	Corn flour 46g/L, Soybean Cake Powder 35g/L, Glucose 1g/L, KH_2PO_4 3.2/L, $MnSO_4$ 0.1g/L,
Media No.13	Soluble starch 3g/L, Soybean Cake Powder 30g/L, KH_2PO_4 1.5g/L, K_2HPO_4 3g/L, Mn SO_4 2g/L
Media No.14	Corn flour 20g/L, Soybean Cake Powder 30g/L, Wheat bran 20g/L, Sodium Citrate 5g/L, KH_2PO_4 0.3/L, K_2HPO_4 1g/L, $CaCl_2$ 1g/L
Media No.15	Urea 2.5g/L, Peptone 2.5g/L, Wheat bran 50g/L, NaCl 5g/L, K_2HPO_4 1g/L, $MnSO_4$ 0.2g/L

Second stage juvenile (J_2) of *H. avenae*: Cysts of *H. avenae* were extracted from the infested soil by the wet sieving, sucrose flotation centrifugation methods and kept for four months in the refrigerator at 4°C before hatching (Fenwick, 1940; Riggs *et al*., 2000). Eggs were separated from the cysts by crushing with the help of a rubber cork on a series of three sieves. The sieves measuring as 0.15mm was kept on top followed by 0.05mm middle sieve and 0.038mm was bottom sieve. The eggs were continuous washed with tap water and the bottom sieve was backwashed to collect eggs. Second stage juveniles (J_{2s}) were obtained from eggs kept in sterile distilled water at 15-18°C from 2-10 days and were collected intermittently and frequently for the *in vitro* mortality test.

***In vitro* inhibition assay of *H. avenae*:** Bacterial culture filtrates were tested in 24-well corning cell culture plates. The concentration of *H. avenae* juveniles were adjusted to estimate to about 50 juveniles per 50ml with the ratio of 850ml water (SDW) that was pipetted into each well of the 24-well cell culture plates. Different bacterial culture filtrates (cf) with 100ml of sample was added to 850ml water (SDW) for mortality test of *H. avenae* juveniles in each well of the cell culture plates. Sterile culture medium (L.B) containing 100ml sample was treated as control (ck) treatment for the *H. avenae* mortality assay (Walker *et al*., 1996). The cell culture plates were kept for incubation at 25°C and observed within interval of 12 hours and 24 hours for the motionless juveniles. Bacterial isolate efficacy and performance elucidated significant management and inhibition activity; as the maximum motionless *H. avenae* juveniles appeared in the wells which were treated with different bacterial culture filtrates compared to untreated sterile culture medium (L.B) control

wells. The *H. avenae* cadaver revised mortality was mathematically calculated as under:

Mortality of J_2 (%) = Dead cadaver / Total number of juvenile x 100

Efficacy of bacterial culture filtrates in different media on mortality of *H. avenae* juvenile (J_2): The effects of bacterial culture filtrates produced in different media compositions were tested *in vitro* in 24-well corning cell culture plates against *H. avenae* juvenile (J_2) mortality. *H. avenae* juveniles (J_2) were simultaneously collected from eggs in sterile distilled water at 15-18^{o}C after immediate hatching. Their ratio was adjusted about 80 juveniles per 50µl, 850µl water (SDW) and 100µl of secondary metabolites produced from individual media composition separately. While the sterile culture medium (L.B) containing 100µl sample served as the control for the *H. avenae* mortality assay (Walker *et al*., 1996). After incubation at 25^{o}C of cell culture plates, the observations were recorded with the interval of 12hours and 24hours for the motionless juveniles. The efficacy of second stage juveniles (J_2) were elucidated noteworthy inhibition activity as the maximum motionless *H. avenae* juveniles appeared in the wells which were treated with different secondary metabolites produced in different media compositions compared to untreated sterile culture medium (L.B) control wells.

Effect of different pH on the stability of secondary metabolites: The stability of selected potential bacterial strains of *Pseudomonas* spp. *P. fluorescence* Ps104, *P. fluorescence* Ps109, *P. putida* Ps190, *P. putida* Ps196 and *P. putida* Ps197 culture filtrates efficacy was examined in different pH ranges from acidic 3pH to neutral 7pH and the basic 10pH against J_2 of *H. avenae*. The fresh culture filtrates were obtained in conical flasks (250ml capacity) containing 100ml of Lysogenic Broth (L.B) medium that were inoculated by one ml of the bacterial isolates *viz*., *P. fluorescence* Ps104, *P. fluorescence* Ps109, *P. putida* Ps190, *P. putida* Ps196 and *P. putida* Ps197, then incubated on rotatory shaker GFL Laboratory equipment (120rpm) at 28°C for 24h. The secondary metabolites of each isolate were adjusted to the acidic range by the addition of hydrochloric acid (HCL) and the basic ranges with addition of concentrations of sodium oxychloride (NaOCl) and were kept in refrigerator for 24 hours. Their stability was tested as described earlier and the data was observed accordingly.

Results

Effect of different media compositions on mass culturing of selected bio-control agents: The suitable media composition containing carbon and nitrogen sources were found as media No.9, followed by No. 10, No. 08 and No. 5 for the mass production of selected *Pseudomonas* spp. biocontrol agents (Figs. 1-5) after 100 hours (4days). The optical density of *P. fluorescens* Ps104 significantly noticed and measured as highest in media composition of carbon and nitrogen sources as No.10, followed by media composition No.6. There were no significant difference in bacterial mass production among the media compositions No.5, No.8, No.9 and No.11 having different sources of carbon and nitrogen (Fig. 1).

The optical density of second bacterial strain *P. fluorescens* Ps109 was measured to be significantly the highest in media composition of carbon and nitrogen sources No.9, followed by media composition No.10. The other media compositions; No.5, No.6 and No.8 had no significant differences in mass production having different sources of carbon and nitrogen (Fig. 2).

Similarly the optical density of third bacterial strain *P. putida* Ps190 was measured as the highest significantly in media composition of carbon and nitrogen sources as No.9. However, no significant difference was noticed in the measured quantity of bacterial mass in media composition No.10, No.5, No.6, No.8 and media No.11 after 100 hours (4 days) shaking (Fig. 3).

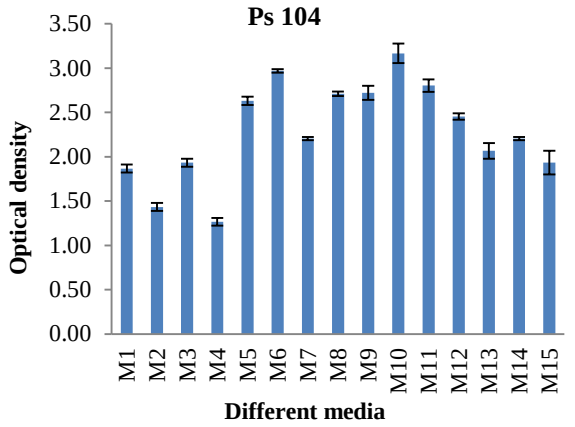

Fig. 1. Media selection for bacterial strain *P. fluorescens* Ps104 mass production.

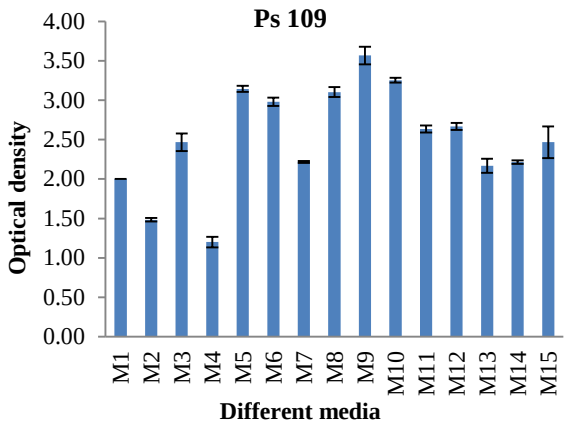

Fig. 2. Media selection for bacterial strain *P. fluorescens* Ps109 mass production.

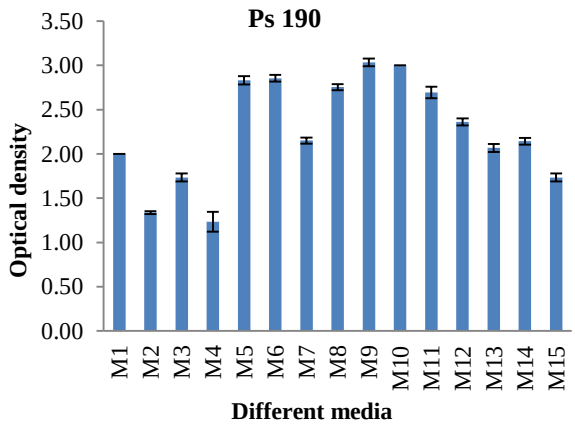

Fig. 3. Media selection for bacterial strain *P. putida* Ps190 mass production.

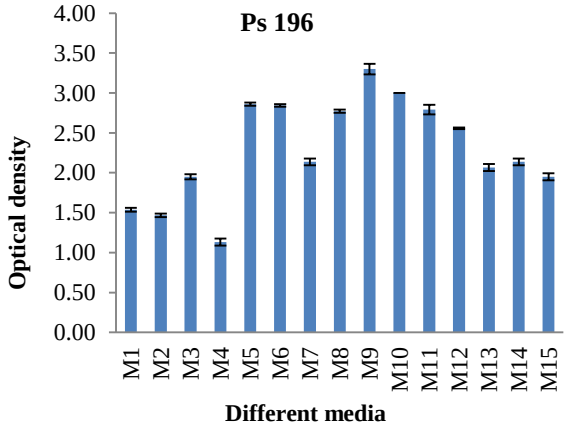

Fig. 4. Media selection for bacterial strain *P. putida* Ps196 mass production.

The optical density of fourth bacterial strain *P. putida* Ps196 was significantly the highest in media composition of carbon and nitrogen sources as No.9. However, no significant differences were observed in the measured quantity of bacterial mass in media composition No.5, No.6, No.8, No.10 and media No.11 after 100 hours (4 days) shaking (Fig. 4).

The optical density of fifth bacterial strain *P. Putida* Ps197 was significantly the highest in media composition of carbon and nitrogen sources as No.9. However, there were no significant differences in the measured quantity of bacterial mass in media composition No.5, No.6, No.10 and media No.11 after 100 hours (4 days) shaking (Fig. 5).

***In vitro* effect of different bacterial culture filtrates on *H. avenae* juveniles (J_{2s}):** The bacterial culture filtrates produced in different suitable media compositions showed varied type of efficacy of toxins against the *H. avenae* juveniles (J_2) during the different exposure time periods (Figs. 6-10). The culture filtrates of bacterial strain *P. fluorescens* Ps104, that were formed during fermentation process and were extracted from media No.5 showed highest mortality of *H. avenae* juvenile (J_2) during initial 12 hour exposure time and the subsequent

duration of 24 hour exposure time followed by secondary metabolites formed during fermentation and extracted from media No.8 as compared to the other media compositions and control treatment (Fig. 6).

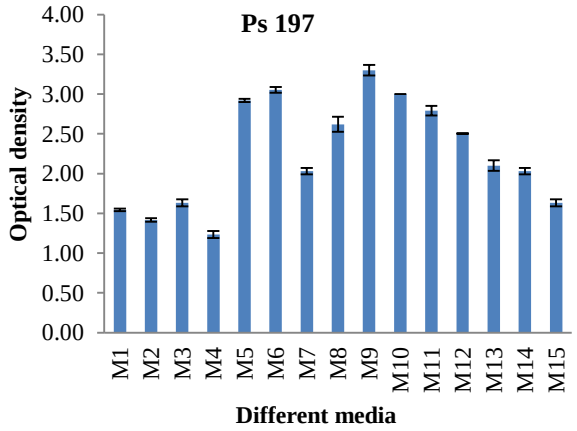

Fig. 5. Media selection for bacterial strain *P. putida* Ps197 mass production.

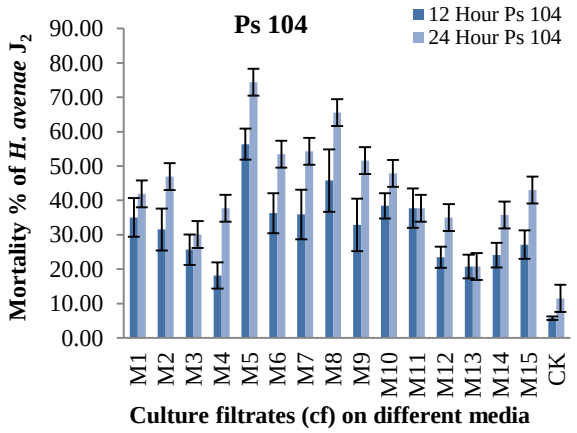

Fig. 6. Effects of individual bacterial culture filtrates produced during fermentation from bacterial strain *P. fluorescens* Ps104 on mortality of second stage juveniles of *H. avenae*.

After fermentation and extracted bacterial culture filtrates of bacterial strain *P. fluorescens* Ps109 from media No.2 showed highest mortality of *H. avenae* juveniles (J_2) during initial 12 hours exposure time and the subsequent duration of 24 hours exposure time followed by secondary metabolites formed during fermentation and extracted from media No.3, No.5 and No.8 as compared to the other media compositions and control treatment (Fig. 7).

The extracted culture filtrates of bacterial strain *P. fluorescens* Ps190 from media No.5 showed highest mortality of *H. avenae* juveniles (J_2) during initial 12 hour exposure time and the subsequent duration of 24 hour exposure time followed by culture filtrates formed during fermentation process and extracted from media No.2, No.3, No.7 and No.8 as compared to the other media compositions and control treatment (Fig. 8).

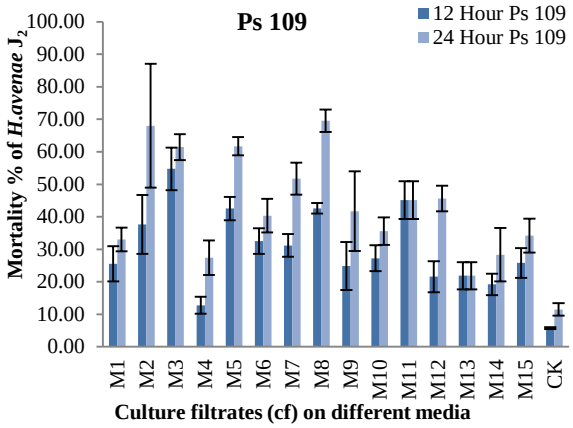

Fig. 7. Effects of individual bacterial culture filtrates produced during fermentation from bacterial strain *P. fluorescens*Ps109 on mortality of second stage juveniles of *H. avenae.*

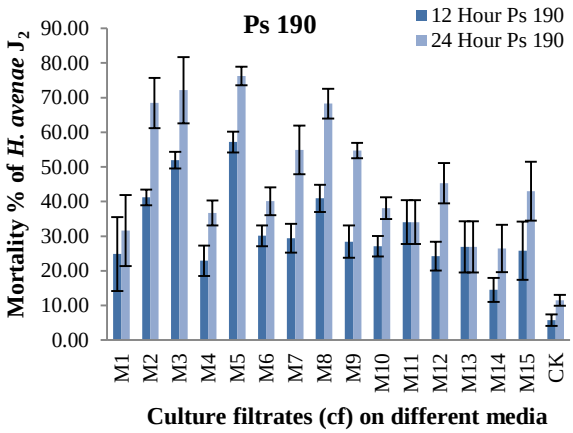

Fig. 8. Effects of individual bacterial culture filtrates produced during fermentation from bacterial strain *P. putida* Ps190 on mortality of second stage juveniles of *H. avenae*.

The extracted bacterial culture filtrates of bacterial strain *P. fluorescens* Ps196 from media No.3 showed highest mortality of *H. avenae* juveniles (J_2) during initial 12 hour exposure time and the subsequent duration of 24 hour exposure time followed by culture filtrates formed during fermentation process and extracted from media No.2 and No.5 as compared to the other media compositions and control treatment (Fig. 9).

The highest *H. avenae* juvenile (J_2) mortality was observed by extracted bacterial culture filtrates of bacterial strain *P. fluorescens* Ps197 from media No.8 during initial 12 hour exposure time and the subsequent duration of 24 hour exposure time followed by culture filtrates formed during fermentation process and extracted from media No.5 and No.2 as compared to the other media compositions and control treatment (Fig. 10).

Effect of different pH on stability of bacterial culture filtrates: The stability of bacterial culture filtrates produced by different bacterial isolates of *Pseudomonas* spp. showed varied type of results for their efficacy on the J_2 *H. avenae* mortality *in vitro* during 12 hour time duration and 24 hour exposure time (Figs. 11-15). The culture filtrates produced by *P. fluorescens* Ps104 showed stability on pH between the pH4 acidic to pH8 basic and had significant effect on the mortality of *H. avenae* juveniles as compared to other ranges of pH and control treatment during 12 hour and 24 hour exposure time (Fig. 11).

Additionally, it was observed that the bacterial culture filtrates produced by *P. fluorescens* Ps109 had stability between the pH3 acidic to pH10 basic and had significant effect on the mortality of *H. avenae* juveniles as compared to other ranges of pH and control treatment during initial 12 hour exposure time, but found reduction in subsequent exposure time period for 24 hour time duration as compared to the control treatment (Fig. 12).

Moreover, it was noticed that the culture filtrates produced by *P. putida* Ps190 had stability between the pH5 acidic to pH8 basic and had significant effect on the mortality of *H. avenae* juveniles as compared to other ranges of pH and control treatment during initial 12 hour and 24 hour time duration as compared to the control treatment (Fig. 13).

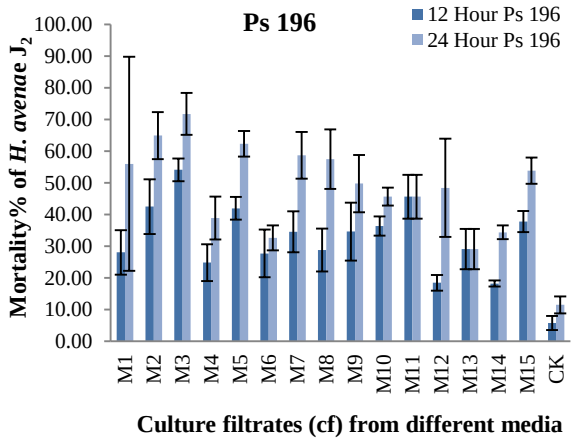

Fig. 9. Effects of individual bacterial culture filtrates produced during fermentation from bacterial strain *P. putida* Ps196 on mortality of second stage juveniles of *H. avena*.

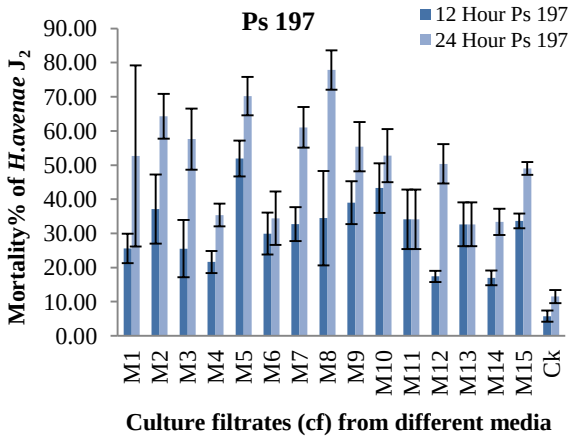

Fig. 10. Effects of individual bacterial culture filtrates produced during fermentation from bacterial strain *P. putida* Ps197 on mortality of second stage juveniles of *H. avenae.*

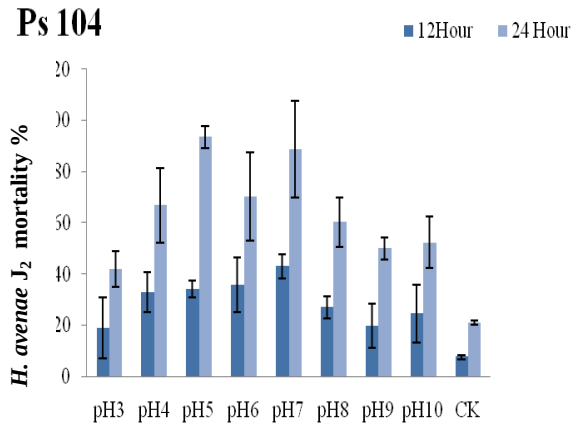

Fig. 11. Effects of pH ranges on bacterial culture filtrates stability produced from bacterial strain *P. fluorescens* Ps104 on mortality of second stage juveniles of *H. avenae*.

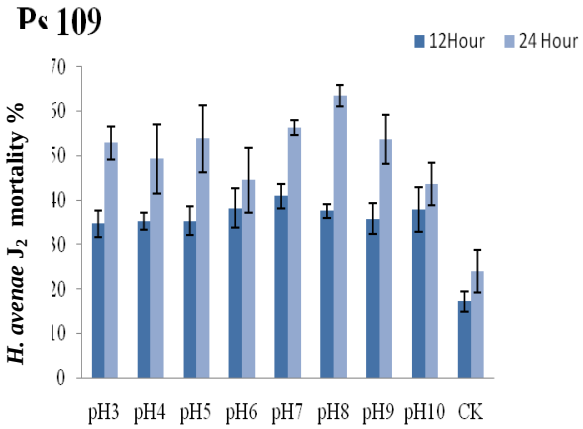

Fig. 12. Effects of pH ranges on bacterial culture filtrates stability produced from bacterial strain *P. fluorescens* Ps109 on mortality of second stage juveniles of *H. avenae.*

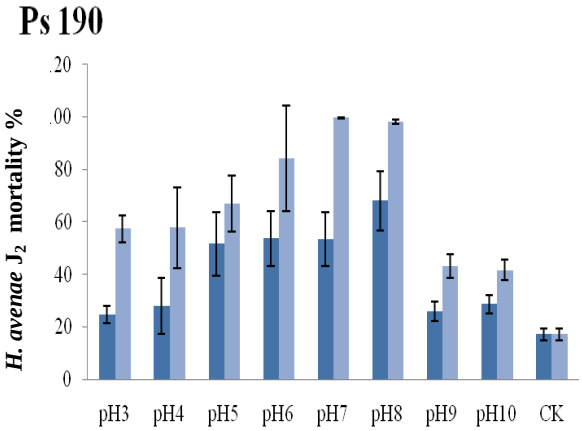

Fig. 13. Effects of pH ranges on bacterial culture filtrates stability produced from bacterial strain *P. putida* Ps190 on mortality of second stage juveniles of *H. avenae*.

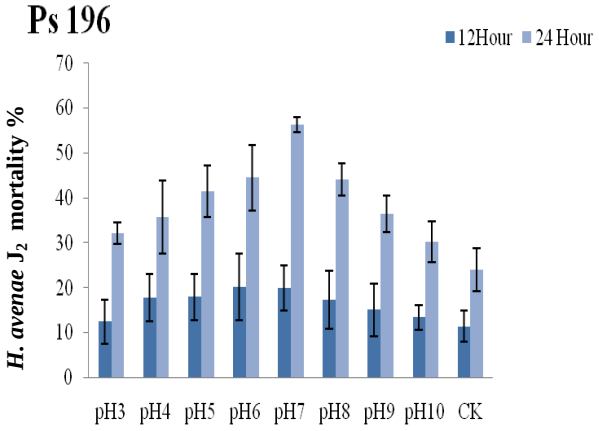

Fig. 14. Effects of pH ranges on bacterial culture filtrates stability produced from bacterial strain *P. putida* Ps196 on mortality of second stage juveniles of *H. avenae*.

Furthermore, it was clearly observed that the culture filtrates produced by *P. putida* Ps196 had stability on the range of pH between the pH3 acidic and pH7 neutral and towards pH10 basic and had significant effect on the mortality of *H. avenae* juveniles when compared to control treatment during initial 12 hour exposure time period. The stability of culture filtrates declined with the subsequent exposure time duration of 24 hour time duration as compared to the control treatment (Fig. 14).

The bacterial culture filtrates produced by *P. putida* Ps197 had stability between the pH4 acidic to pH8 basic and had significant effect on the mortality of *H. avenae* juveniles as compared to other ranges of pH and control treatment during initial 12 hour and 24 hour time duration as compared to the control treatment (Fig. 15).

Discussion

The biological control of diseases and pests is the alternate control measure of pathogens, especially

the soil-borne nematodes. The individual contribution or product formulation should be clearly outlined or mentioned as it is essential for product registration and the production cost. However, the environmental factor that regulates the growth and bio-control efficacy of antagonist bacteria is an essential step towards advancing the parameters and reliability of their potentiality (Duffy & Defago, 1999). The isolation from environmental niche of potential isolates, it's *in vitro* screening in suitable environmental conditions with association to particular pathogen and stability during application are the crucial stages which may determine the successes or failure of putative product.

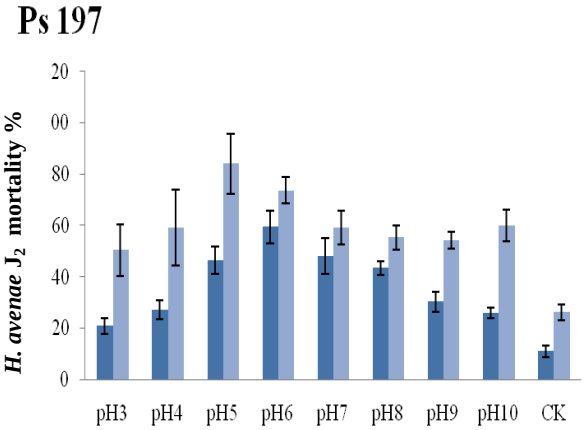

Fig. 15. Effects of pH ranges on bacterial culture filtrates stability produced from bacterial strain *P. putida* Ps197 on mortality of second stage juveniles of *H. avenae*.

In this study the genus *Pseudomonas* was investigated which belongs to the subclass of the *Proteobacteria* and includes mostly florescent *Pseudomonades* as well as non-florescent species. *P. fluorescence* and *P. putida;* which were more efficient than other species against different soil-borne pathogens and nematodes of different cereal crops. Both these species showed varied mechanism viz., they directly start the lethal biological process and can enhance the growth promoting actions or may only suppress the bio aggressor by competition. They also induce the resistance factors in plants. *P. putida* and *P. fluorescence* potential strains are able to improve plant growth and health and caused inhibition of cereal cyst nematode during the J_2 stage. The fermentation techniques, formulations and production methods yielded high number of cell and the isolate was shown to have retained its secondary metabolites activity or property after preservation and bench scale production based *in vitro* inhibition of *H. avenae* infective juvenile (J_2). The suitable media compositions should therefore be carefully studied and optimized as the recipe used may have cost implications in mass production techniques. In this experiment, we investigated some aspects so that evidence can be provided for the byproducts to become more integrated into management strategies for protection and curative measures for the menace of soil-borne cereal cyst nematode (CCN) *H. avenae*. This study has shown that antagonistic bacteria such as *P. fluorescence* and *P. putida* can be produced in different media, using carbon and nitrogen sources while maintaining the efficacy as bio-control of soil-borne cereal cyst nematode *H. avenae in vitro* and in field.

The entomopathogenic bacterium *Pseudomons* spp., and its toxic secretions were used for the insect control which is a significant component of integrated pest management (IPM) against different pests (Mahar *et al*., 2005). It also enhances the plant growth and causes significant inhibitory effect on the hatching of nematodes and penetration in the roots (Siddiqui *et al*., 2007). There are some studies examining the mechanisms of the actions. Following mechanisms of actions are thought to be responsible for reduction in nematode infection: production of secondary metabolites for reduction in hatch and attraction; degradation of specific root exudates which manage the nematode behavior; enhancement of the defense mechanism in plants leading to the induction of systemic resistance (ISR) (Sikora& Hoffmann-Hergarten, 2008; Hallmann *et al*., 2001; Siddiqui *et al*., 2001).

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