

CHAPTER VII
PERMEABILITY AND ANTIMICROBIAL ACTIVITIES OF
POLYPROPYLENE NANOCOMPOSITE FILMS

7.1 Abstract

The primary objective is to develop PP-clay nanocomposite films to enhance the gas barrier property and antifungal activity required for food packaging among other applications. The effect of modified silver nanoparticle-loaded bentonite (MSBEN) contents on gas permeability and antifungal activity of polypropylene-clay nanocomposites have been studied. The water vapor permeability rate (WVPR) and oxygen transmission rate (OTR) of nanocomposite films were investigated in accordance to ASTM E398 and ASTM D3985, respectively. As for antifungal activity of antifungal agent immobilized plastic films, the test was carried out by agar disc diffusion assay, according to ASTM G 21. This work agrees with the use of given clay (MBEN, MS5BEN, MS10BEN, MS15BEN, MS20BEN) increased the mobility distance of the gas molecules, bringing about the water and oxygen permeability reduced by 77 % and 33 % in the case of PPBEN compared to neat PP. Furthermore, in the presence of silver nanoparticles, both water and oxygen permeability rate decreased by approximately 70% and roughly 20% in all the PPSBEN films in comparison to neat PP films. From the antifungal activity test, all the S5BEN, S10BEN, S15BEN, and S20BEN can inhibit a growth of fungi, *Colletotrichum gloeosporioides*, after 1 day of antifungal screening test. This result implies the material is an interesting choice for a smart packaging.

Keywords: Antifungal, *Colletotrichum gloeosporioides*, Nanocomposites, Permeability, Polypropylene

7.2 Introduction

The potential use of plastics in the packaging industry has gained enormous researchers' attention because of their lightweight, inexpensive, disposable, and design flexible. Nevertheless, polymer materials are permeable to gases, such as oxygen, carbon dioxide, and water vapor, and organic materials and commercial usages are limited in certain cases. In the polymer-clay nanocomposites, the presence of modified clay is anticipated providing a gas barrier resistance because the clay layers are impermeable to gases by creating a lot of tortuous paths. These paths led to a decrease in permeability of water and oxygen.

Furthermore, in order to enhance the antimicrobial property of packaging films, the silver nanoparticle is one kind of antimicrobial agents extensively incorporated in the films at present. As silver nanoparticles can disrupt the enzymatic activity of microbial cells, one important aim of addition of these metallic silver nanoparticles is to prolong the shelf-life and promote the safety of meat products by reducing the rate of surface growth of specific microorganisms by direct contact of the packaging with the products (Appendini and Hotchkiss, 2002).

In this section, each masterbatch of PP mixed with modified bentonite and PP and PP mixed with modified silver nanoparticles-loaded bentonite was prepared via plasma process. The as-prepared masterbatch was then mixed with PP in order to obtain PP nanocomposites containing 1 wt% of modified clay. After that, the nanocomposite films was carried out by a blown film extrusion machine to produce a clear film for food packaging application. The effects of contents of silver nanoparticles (5, 10, 15 and 20 wt%) on the barrier and antimicrobial activity of PP nanocomposite blown films were determined and reported in this chapter.

7.3 Experimental Parts

7.3.1 Materials

Commercial polypropylene (PP) under trade name 2300K (MFI 4 g/10 min) supported from T.H.L. Industry Co., Ltd. was used as received. Commercial polypropylene (PP) under trade name NK 1126 (MFI 11 g/10 min)

supported from T.H.L. Industry Co., Ltd. was used as received. Dicumyl peroxide (DCP), CAS NO. 80-43-3 was purchased from Aldrich and used as received. The commercial sodium activated bentonite Mac-Gel[®] (GRADE SAC), Na-BTN, with cationic exchange capacity (CEC) of 49.74 meq/100 g clay and surface area of 31.0 m²/g, supplied by Thai Nippon Co., Ltd. Thailand, was purified before use. Silver (I) nitrate (AgNO₃), CAS NO. 7761-88-8, was purchased from Carlo Erba and used as received. Trisodium citrate dihydrate (Na₃C₆H₅O₇), CAS NO. 6132-04-3, was purchased from Carlo Erba and used as received. Absolute ethanol (C₂H₅OH), AR grade, CAS NO. 64-17-5, and glacial acetic acid (CH₃COOH), CAS NO. 64-19-7, were purchased from RCI Labscan and used as received, respectively

7.3.2 Preparation of Purified Clay

The sodium bentonite clay was first vigorously stirred into a deionized water at 700 rpm overnight by a mechanical stirrer. After that, the supernatant (swollen clay) was separated by centrifugation at 10,000 rpm for 15 minutes and lastly dried, sieved by mesh#400 and kept in a dessiccator before use.

7.3.3 Modification of Bentonite

1.5 wt.% of MPS solution was prepared in 70 vol.% of ethanol aqueous solution pH 4 adjusted by glacial acetic acid and then stirred for 45 min of hydrolysis to silanol. 50 g of bentonite were added respectively into MPS solution. The mixture was stirred at 110 °C for 24 hr. The plenty of absolute ethanol was used for the removal of excessive MPS during suction filtrating and the modified clay was finally dried at 80 °C in vacuum oven for 24 hr. The clay was pulverized by ball mill and then sieved by mesh#400 and kept in a desiccator prior to use.

7.3.4 Preparation of Silver Nanoparticle-Loaded Clay (SBEN)

50 g of purified bentonite were firstly stirred in AgNO₃ solution at room temperature overnight. The silver-ion clay was then reacted with C₆H₅Na₃O₇·2H₂O at 1 : 3 molar ratio of AgNO₃ to C₆H₅Na₃O₇·2H₂O at 90 °C for 20 min to obtain a silver nanoparticles-loaded clay (SBEN). The as-synthesized silver nanoparticles-loaded clay was measured their shape and size by a transmission electron microscope (TEM) as well as their crystal structure by an X-ray diffractometer (XRD).

7.3.5 Modification of Silver Nanoparticles-Loaded Clay (MSBEN)

The as-prepared silver nanoparticles-loaded bentonite (SBEN) was modified via silanation process. 1.5 wt.% of MPS solution was prepared in 70 vol.% of ethanol aqueous solution pH 4 adjusted by glacial acetic acid and then stirred for 45 min of hydrolysis to silanol. 50 g of the clay were added respectively into the MPS solution. The mixture was stirred at 110 °C for 24 hr. The plenty of absolute ethanol was used for the removal of excessive MPS during suction filtrating and the modified silver nanoparticles-loaded bentonite (MSBEN) was finally dried at 80 °C in vacuum oven for 24 hr. The clay was pulverized by ball mill and then sieved by mesh#400 and kept in a desiccator prior to use.

7.3.6 Preparation of Polypropylene-Modified Bentonite Masterbatch and Polypropylene-Modified Silver Nanoparticles-Loaded Bentonite Masterbatch via a Plasma-based Processing

Both the PP and MBEN (7.3.3) and PP and MSBEN (7.3.5) were mixed in the co-rotating twin-screw extruder (Labtech types LTE-20-32 & LTE-20-40 screw diameter 20 mm) with L/D ratio 40:1. The operation temperature was carried out at 100, 180, 185, 190, 190, 190, 195, 200, and 200 °C from hopper to die, respectively and the screw speed at 10 rpm. During exiting die, the melt extrudate of each of the compounds was immediately treated by the plasma generator (PT-1 PLASMA TREATER) operated at 6 kV, 10 kHz for 5 sec at the distance of 4 cm from its nozzle to the extrudate.

7.3.7 Preparation of Active Films

The masterbatch of PP-modified bentonite (PPC-PLASMA) or PP-MSBEN was further mixed with PP pellet in the ratio of PP : modified clay = 99 : 1 wt% (each compounds containing 1 wt.% of modified clay) in the co-rotating twin-screw extruder (Labtech types LTE-20-32 & LTE-20-40 screw diameter 20 mm) with L/D ratio 40:1. The operation temperature was performed at 100, 180, 185, 190, 195, 195, 195, 195, 200, and 200 °C from hopper to die, respectively and the screw speed at 30 rpm. Each of the nanocomposites was transformed into active films by using a Labtech (model LTE20-30) single-screw extruder with L/D = 40 (D = 20 mm and L = 800) and annular die with outer diameter of 70 mm and inner diameter of 68.5 mm. The operating temperature of blown film extrusion machine was operated

in the range of 180 – 200 °C and the rotational screw speed was fixed at 60 rpm with the blow up ratio of 2 : 1.

7.3.8 Characterization

7.3.9.1 *Gas Permeability Testing*

Vapor Permeation Tester Model L80-4000, LYSSY was used to determine water vapor permeability of neat PP and PP nanocomposite films. Water vapor permeation experiments were investigated following procedure described in ASTM E398. The test was performed at 38 °C with water vapor pressure of 49.7 mmHg. The blown films were cut into circular shape with 15 cm in diameter. The thickness of films was measured using peacock digital thickness gauge PDN 12N by reading 15 points at random position over test area.

Oxygen Permeation Analyzer Model 8500, Illinois Instrument Inc., was used to determine oxygen permeability of neat PP and PP nanocomposite blown films. Gas permeation experiments were investigated following procedure described in ASTM D3985. The test was carried out at 25 °C with oxygen flow rate of 40 cm²/min. The blown films were cut into circular shape with 11.3 cm in diameter. The thickness of films was measured using peacock digital thickness gauge PDN 12N by reading 15 points at random position over test area.

7.3.9.2 *Antifungal Screening Test of Active Films*

The standard test method (ASTM G 21) for assessing the antifungal activity of immobilized antifungal agents (silver nanoparticles) under qualitative determination, called agar-based disc diffusion assay, was also applied for providing a straightforward measurement of the intrinsic fungal resistant property in antifungal agent immobilized clay in comparison to modified clay without silver nanoparticles and such a test also applied for investigating the antifungal property in PPSBEN films compared to control films which are the virgin PP and PPBEN films.

7.3.9.2.1 Preparation of Fungal Strain

The strains of fungi were obtained from Food Science and Technology department, Chiang Mai University. *Colletotrichum gloeosporioides* (*C. gloeosporioides*, identified by Department of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University)

were used for testing antifungal activity. The species of fungus were grown in potato dextrose media. The concentration of fungal suspensions was adjusted to 10^{-6} CFU.

7.3.9.2.2 *Antifungal Assay* (Ghosh *et al.*, 2011)

The intrinsic fungal resistant property in both silver nanoparticles immobilized clays and antifungal agent immobilized films, antifungal activity was also measured using the method of diffusion disc plates on agar.

For antifungal activity measurement of silver nanoparticles immobilized clays, the fungal suspensions of 10^{-6} CFU were spread onto the agar plates as explained above. The agar was drilled as a hole (not exceeding 0.2 mm) for nanoclays. The 0.1 mg of each nanoclays was then placed into the hole. Eventually, the inhibition diameters were determined after incubation at 27 °C for 24 and 48 hours. All tests were made in triplicates.

For antifungal activity measurement of silver nanoparticles immobilized nanocomposite films, the fungal suspensions of 10^{-6} CFU were spread onto the agar plates as explained above. Each of the nanocomposite films was cut into circular shape with diameter of 2 cm and was then placed cover the center of agar plates. Eventually, the inhibition diameters were determined after incubation at 27 °C for 24, 48, 36 hours, and 7 days. All tests were made in triplicates.

For antifungal activity measurement, which are adapted by Suphat Kumthai from Department of Packaging Technology, Faculty of Agro-Industry, Chiang Mai University), of silver nanoparticles immobilized nanocomposite films, each of the nanocomposite films was cut into circular shape with diameter of 2 cm and was then placed into the center position of agar plates. The matured fungal mold at the age of 7 days was cut into rectangular shape with $1 \times 1 \text{ cm}^2$ and then placed onto 4 positions around nanocomposite films. Eventually, the inhibition diameters were determined after incubation at 27 °C for 24 hours. All tests were made in triplicates.

7.4 Results and Discussion

7.4.1 Water Vapor Permeability Rate (WVPR) and Oxygen Transmission Rate (OTR) of Neat PP and Corresponding Nanocomposite Films

The transport properties are known to be strongly affected by tortuous path altering factors including aspect ratio and shape of the filler, degree of exfoliation or dispersion, filler loading (volume fraction) and orientation, adhesion between filler and matrix, moisture activity, filler-induced crystallinity, polymer chain immobilization, filler-induced solvent retention, degree of purity, porosity, and size of the permeant (Villaluenga *et al.*, 2007).

In the Figure 7.1 and Figure 7.2, it can be vividly observed that the blown nanocomposite films significantly exhibited lower WVPR and OTR than those of the neat PP film. Figure 7.1 shows a decrease in WVPR in all nanocomposite films with the addition of the MBEN and MSBEN at all loading levels of silver nanoparticles compared to the neat PP film. The WVPR of PPBEN was 0.62, whereas the WVPR of the PPS5BEN, PPS10BEN, PPS15BEN, and PPS20BEN were only 0.88, 0.79, 0.80, and 0.77, respectively. For OTR measurement, the blown PPMBEN had an OTR of 3580 cc/m²/day, 33% lower than the neat PP film, which had an OTR of 5323 cc/m²/day. A slight increase in WVPR and OTR was observed with the addition of the silver nanoparticles. For example, in the case of PPS5BEN, the neat PP film had WVPR and OTR of 2.74 g/m²/day and 5323 cc/m²/day, respectively, while the PPS5BEN film had WVPR and OTR of 0.88 g/m²/day and 3935 cc/m²/day. However, generally the permeation rates of both the blown nanocomposite films remained nearly constant with the incorporation of the silver nanoparticles. This implied that no significant reduction in the water vapor and oxygen barrier properties of the resultant films. To conclude, the improvement of gas barrier properties can be attributed to the more tortuous path created to retard the permeability rate of gas diffusion through all of the nanocomposite films (Seephueng, 2008). Moreover, in her work, Seephueng (2008) suggested that another possible reason for the reduction in both of the WVPR and OTR was that the higher crystallinity restricted the chain mobility concerned with the diffusion process of gas. The latter reason agreed with the work because after adding the MBEN and all the

MSBENs, the DSC measurement showed the higher percentage of crystallinity due to nucleating effect from the clay and the silver nanoparticles. As a result, the WVPR and OTR were reduced compared to the neat PP film. Moreover, in association with the morphology of the nanocomposite films, it was, unexpectedly, further observed that in the case of PPSBENs, their WVTR and OTR values were increased a little bit than those of PPBEN corresponding to the bigger voids presented in the SEM images caused by the inhomogeneous fillers in the PP matrix stemming from the voids formed at or near the interface between polypropylene matrix and nanoclay during the blown film extrusion process. Another possible reason stemmed from certain degrees of hydrophilicity at the surface of the silver nanoparticles within the clay intergalleries, which resulted from the hydrated reducing agent and stabilizer (trisodium citrate dihydrate) coated on the surface of the silver nanoparticles.

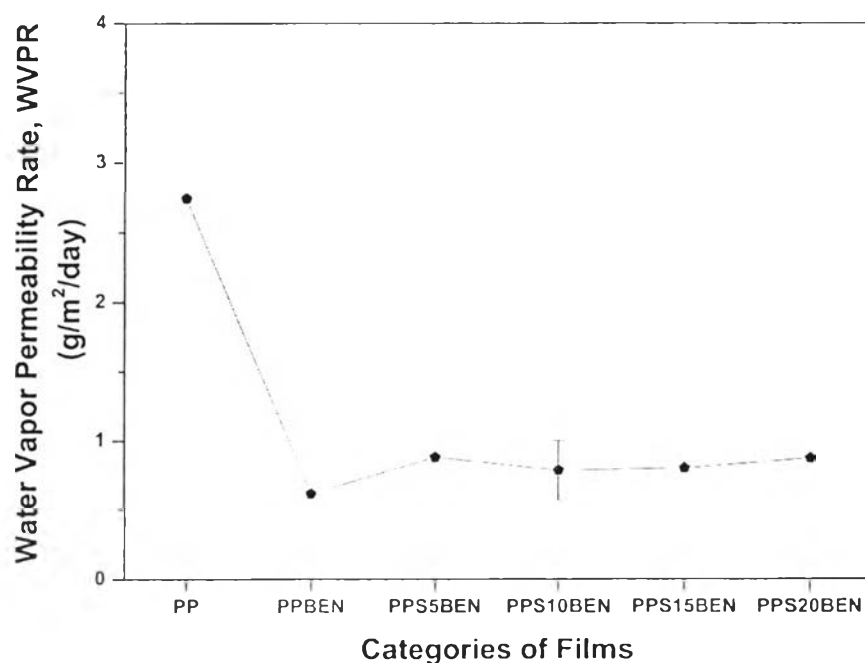


Figure 7.1 Water vapor permeability rate of neat PP and corresponding nanocomposite films.

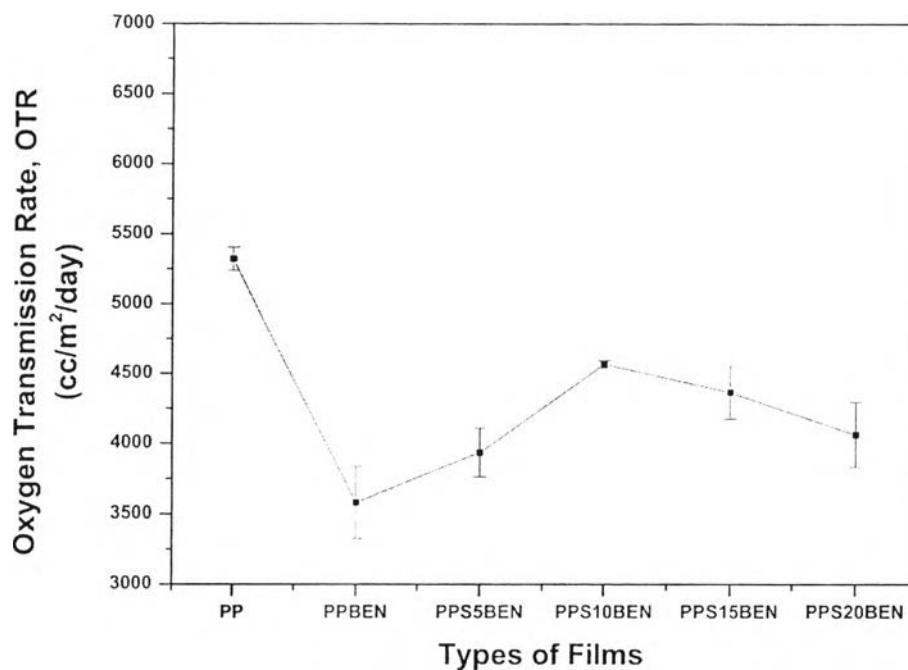


Figure 7.2 Oxygen transmission rate of neat PP and corresponding nanocomposite films.

7.4.2 Antifungal screening test of silver nanoparticles-loaded bentonites and nanocomposite films against *Colletotrichum gloeosporioides* (*C. gloeosporioides*)

The antifungal test (Figure 7.3 (a-e) and 7.4 (a-e)) pointed out that the silver nanoparticles added bentonites demonstrated the clear inhibition zones as circular shapes around the silver nanoparticles added bentonites. The clear inhibition zones were evidently speculated after 2 days of incubation, especially S20BEN, the clay with 20 wt% of silver nanoparticles, showed the widest clear zone which implied that the maximum resistance to silver nanoparticles going along with the highest contents of antifungal agents in this experiment. Therefore, the silver nanoparticles releasing from the bentonite are influential in inhibiting the growth of *Colletotrichum gloeosporioides*.

The antifungal revealed that MBEN cannot inhibit the fungi growth after 2 days of the test. It was speculated by the turbidity of these films resulting from the very high density fungal population at the film border. On the other hand, all of the SBENs showed their ability to restrain its growth after two days of culture.

However, when the test time reached the second day and lastly third day, the *Colletotrichum gloeosporioides* had tendency to form its spore on the films observed by their turbidity and a tiny white spot within the circular films placed in the agar disc diffusion plates. Nevertheless, based on the researcher's observation at the rim of PPS20BEN, it showed the lowest fungal density around the film, possibly resulted from the diffusion of the highest content of silver nanoparticles at the rim of the film was harmful to fungal cells on the agar plate. However, the low diffusivity of silver ions from the films to agar can inhibit the fungi because of the hydrophobic characteristic of polypropylene by nature.

Furthermore, so as to clearly observe the antifungal activity of the nanocomposite films, the researcher applied a new technique by which the spore of *C. gloeosporioides* at the age of 7 days culture (Figure 7.6) was cut in a little piece. The uniform size of spore piece was placed on the four positions of the agar plate. The circular film ($2 \times 2 \text{ cm}^2$) was placed on the center of agar plate. The plate was incubated at 27°C for 1 day and the result was finally recorded.

The Figure 7.7 (a-f) shows the antifungal activity of all the nanocomposite films (PPS5BEN, PPS10BEN, PPS15BEN, and PPS20BEN) in comparison to neat PP and PPBEN film. The result undoubtedly exhibited the active activity of all the PPSBEN regarding the antifungal films as well as the release of silver nanoparticles against the *C. gloeosporioides* by restraining its growth. Whereas, in the case of the films without added silver nanoparticles, they had a piece of white spore around the film border, which evidently indicated the inactive function of them to against this kind of fungi.

From the Figure 7.6 and 7.7, the antifungal revealed that neat PP and PPSBEN cannot inhibit the fungi growth after 24 hr of the test. It was speculated by the turbidity of these films resulting from the very high density fungal population at the film border. On the other hand, all of the PPSBENs showed their ability to restrain its growth after one day of culture. However, when the test time reached the second day and lastly third day, the *Colletotrichum gloeosporioides* had tendency to form its spore on the films observed by their turbidity and a tiny white spot within the circular films placed in the agar disc diffusion plates. Nevertheless, based on the researcher's observation at the rim of PPS20BEN, it showed the lowest fungal

density around the film, possibly resulted from the diffusion of the highest content of silver nanoparticles at the rim of the film was harmful to fungal cells on the agar plate. However, the low diffusivity of silver ions from the films to agar can inhibit the fungi because of the hydrophobic characteristic of polypropylene by nature.

Furthermore, so as to clearly observe the antifungal activity of the nanocomposite films, the researcher applied a new technique by which the spore of *C. gloeosporioides* at the age of 7 day culture (Figure 7.8) was cut in a little piece. The uniform size of spore piece was placed on the four positions of the agar plate. The circular film ($2 \times 2 \text{ cm}^2$) was placed on the center of agar plate. The plate was incubated at 27°C for 1 day and the result was finally recorded.

The Figure 7.9 (a-f) shows the antifungal activity of all the nanocomposite films (PPS5BEN, PPS10BEN, PPS15BEN, and PPS20BEN) in comparison to neat PP and PPBEN film. The result undoubtedly exhibited the active activity of all the PPSBEN regarding the antifungal films as well as the release of silver nanoparticles against the *C. gloeosporioides* by restraining its growth. Wherease, in the case of the films without added silver nanoparticles, they had a piece of white spore around the film border, which evidently indicated the inactive function of them to against this kind of fungi.

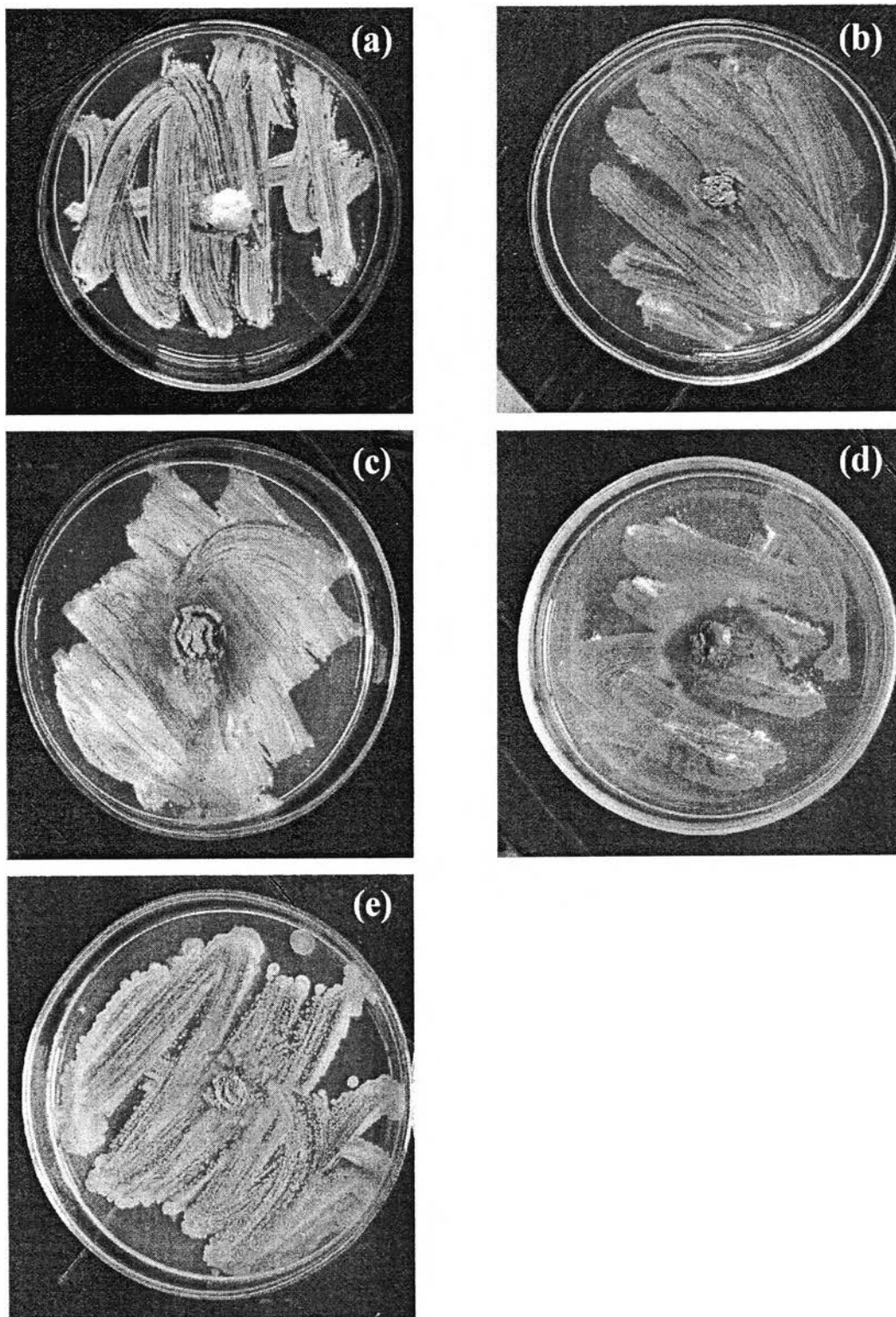


Figure 7.3 Agar disc diffusion test of (a) MBEN (b) S5BEN (c) S10BEN (d) S15BEN (e) S20BEN against *Colletotrichum gloeosporioides* after 1 day of incubation.

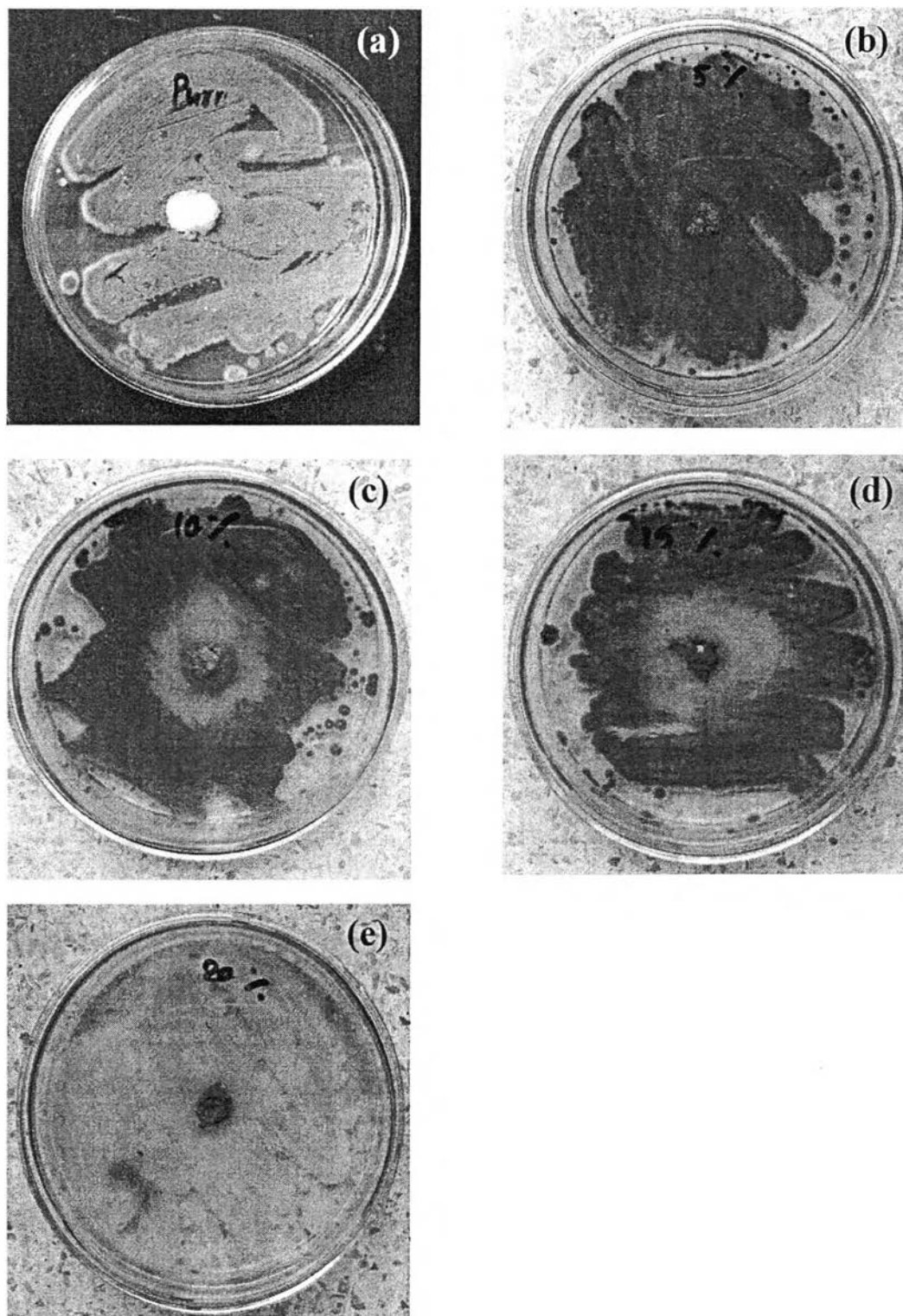


Figure 7.4 Agar disc diffusion test of (a) MBEN (b) S5BEN (c) S10BEN (d) S15BEN (e) S20BEN against *Colletotrichum gloeosporioides* after 2 days of incubation.

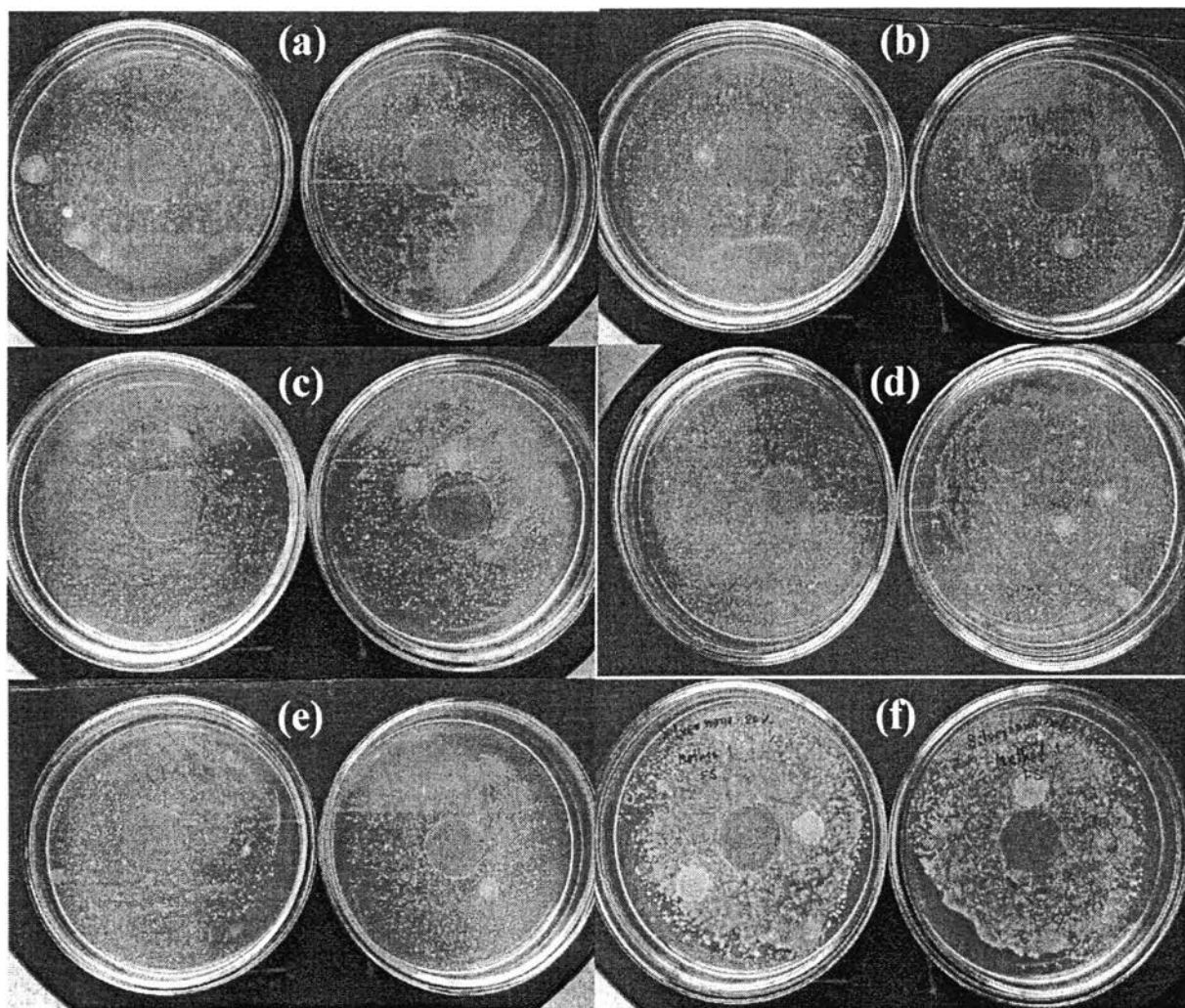


Figure 7.5 Agar disc diffusion test of (a) PP (b) PPBEN (c) PPS5BEN (d) PPS10BEN (e) PPS15BEN (f) PPS20BEN films against *Colletotrichum gloeosporioides* after 1 day of incubation.

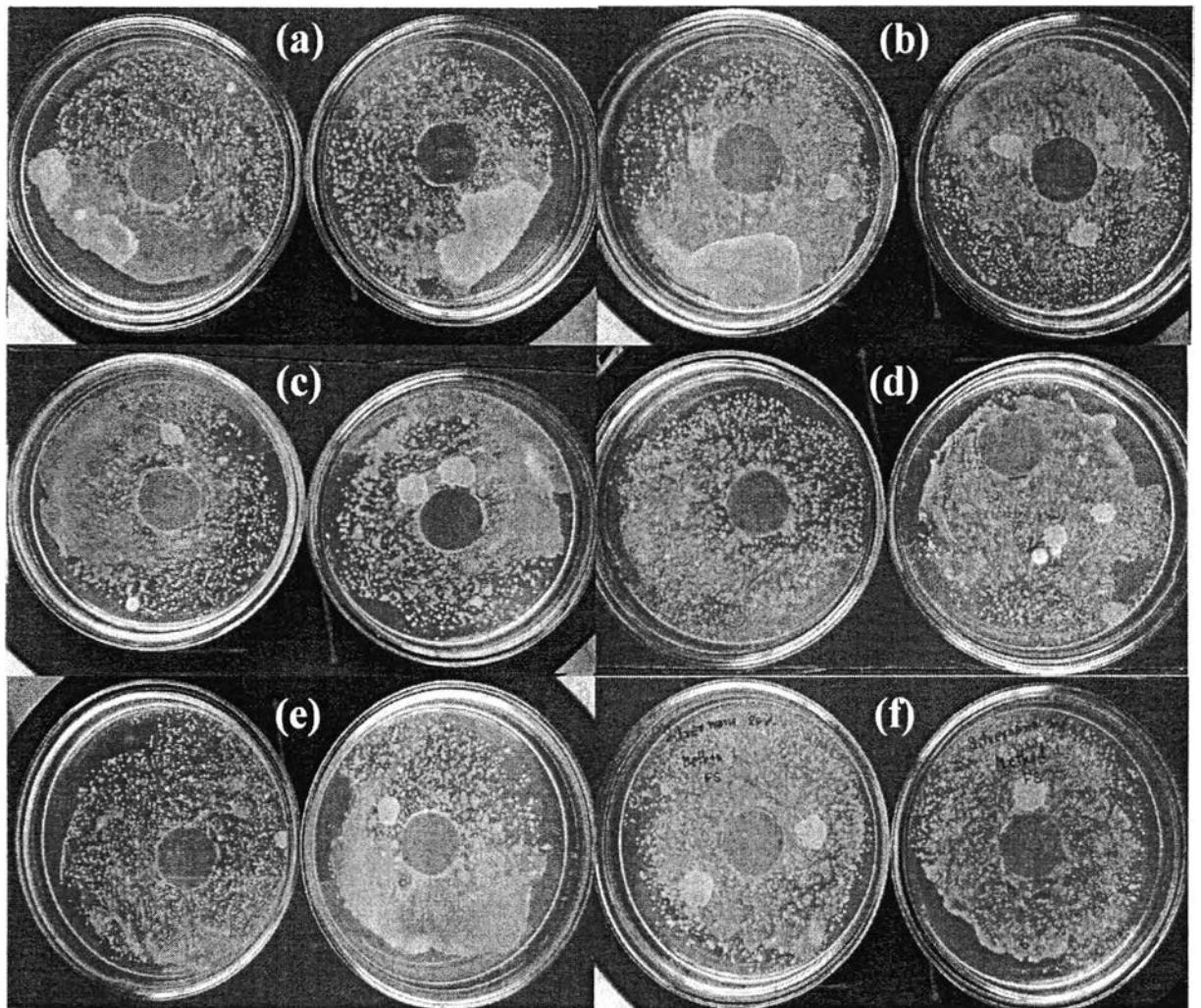


Figure 7.6 Agar disc diffusion test of (a) PP (b) PPBEN (c) PPS5BEN (d) PPS10BEN (e) PPS15BEN (f) PPS20BEN films against *Colletotrichum gloeosporioides* after 2 days of incubation.

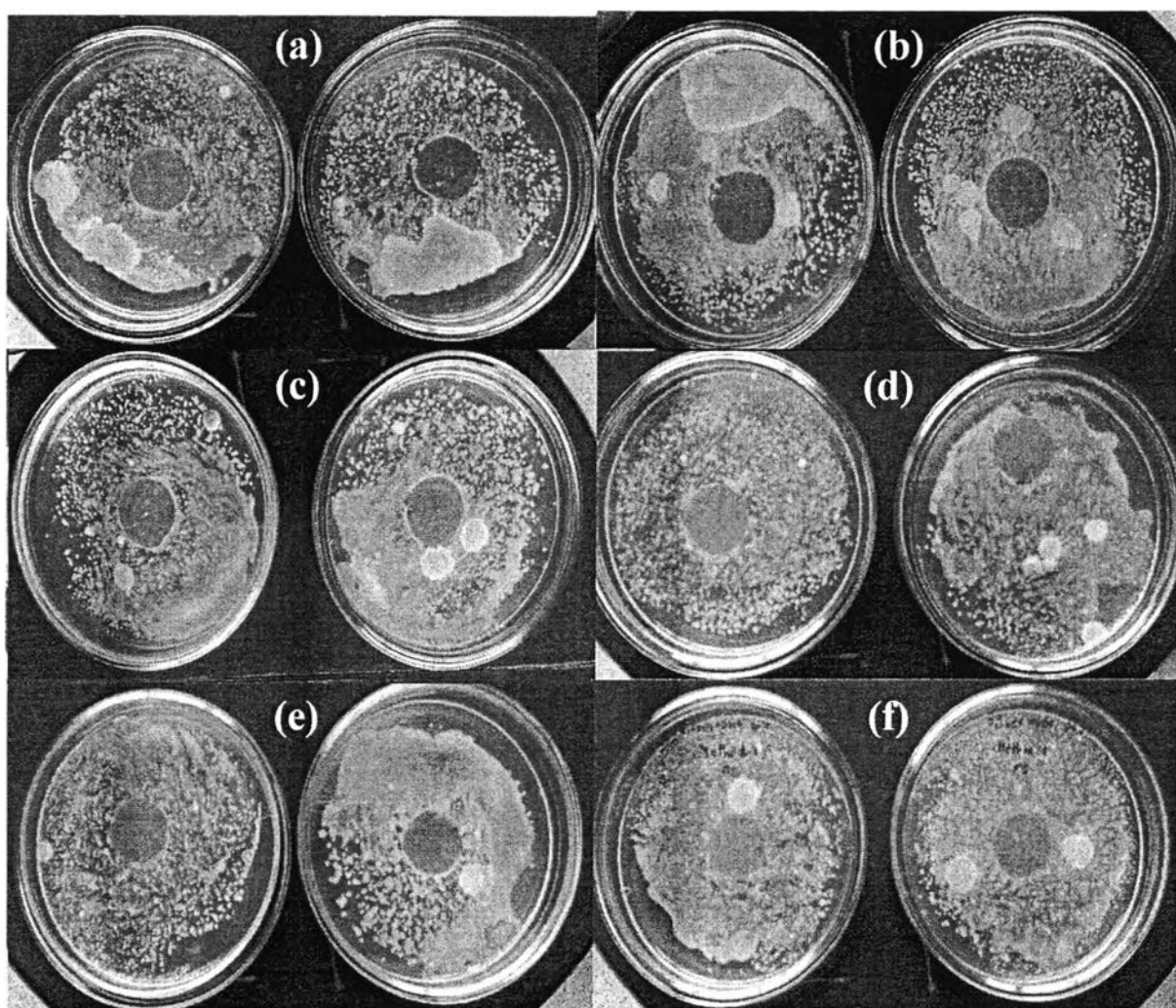


Figure 7.7 Agar disc diffusion test of (a) PP (b) PPBEN (c) PPS5BEN (d) PPS10BEN (e) PPS15BEN (f) PPS20BEN films against *Colletotrichum gloeosporioides* after 3 days of incubation.

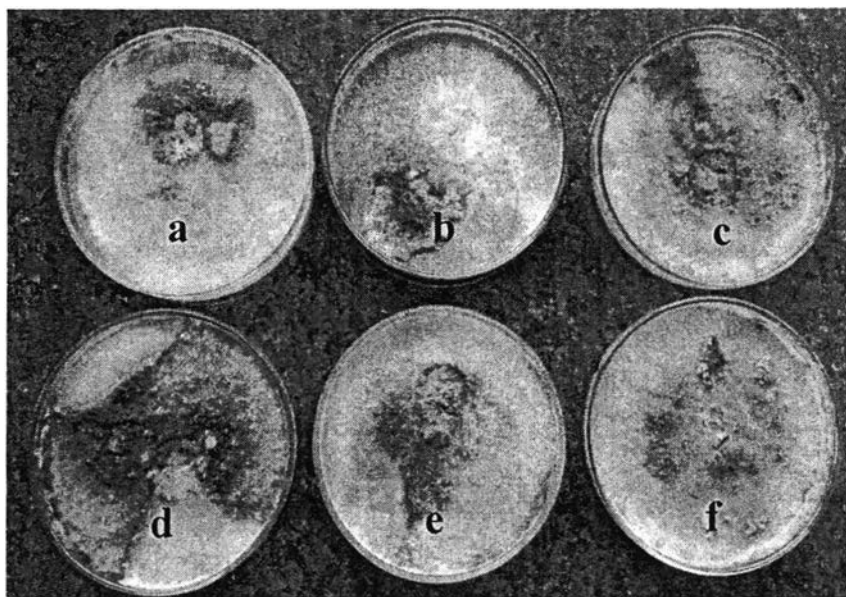


Figure 7.8 Agar disc diffusion test of (a) PP (b) PPBEN (c) PPS5BEN (d) PPS10BEN (e) PPS15BEN (f) PPS20BEN films against *Colletotrichum gloeosporioides* after 7 days of experimental time.

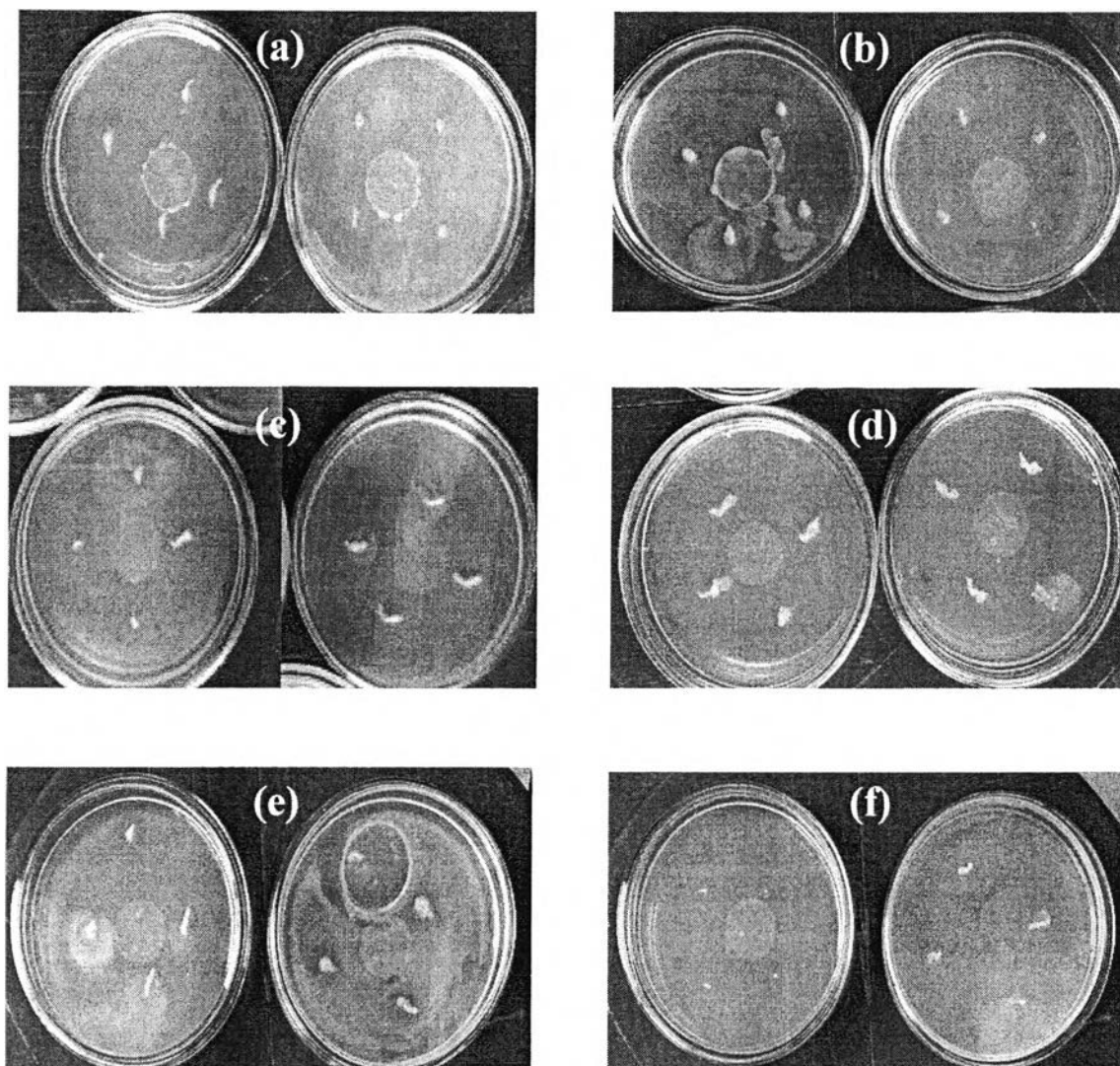


Figure 7.9 Agar disc diffusion test of (a) PP (b) PPBEN (c) PPS5BEN (d) PPS10BEN (e) PPS15BEN (f) PPS20BEN films against *C. gloeosporioides* after 1 day of incubation.

7.5 Conclusions

The incorporation of modified nanoclay had a crucial influence on vapor and gas barrier properties of active films. According to the measurement based upon ASTM E398 and ASTM D3985, the results were evidently observed by a decrease in WVPR and OTR implied that the better barrier improvement. The WVPR and OTR were reduced by 77 % and 33 % in the case of PPBEN compared to neat PP.

Furthermore, in all the cases of PPSBEN films, both water and oxygen permeability rate decreased by approximately 70% and roughly 20% comparable to neat PP films. In addition to the better barrier resistance, PPSBEN importantly exhibited antifungal properties against *Colletotrichum gloeosporioides* (*C. gloeosporioides*) after one day of culture by agar disc diffusion assay accordance to ASTM G 21. However, after three days of the test, it was noticed that the PPS20BEN manifested the strongest antifungal activity to the type of fungi because of the lowest density of fungi growing around the circular PPS20BEN film. To sum up, the improvement of the gas barrier property and antifungal activity of the PP-clay nanocomposite films fulfill the requirement for beverage and food packaging among other applications.

7.6 Acknowledgements

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7.7 References

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