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Full Length Research Paper

Management of post-harvest *Pectobacterium* soft rot of cabbage (*Brassica oleracea* var *capitata* L.) by biocides and packing material

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Bioassay studies of several biocides against *Pectobacterium carotovorum* ssp. c*arotovorum* revealed that ciprofloxacin proved highly inhibitive to bacterial growth by producing highest inhibition zone (21 mm) at a concentration of 200 µg mL^{-1} followed by penicillin, tetracycline, and oxytetracycline. Other test chemicals viz., amoxicillin, striplin, azithromycine, mercuric chloride, bleaching powder, sodium hypochlorite and copper-oxychloride, produced a moderate to low inhibition zone. Studies on use of effective antibiotics for post-harvest management of soft rot revealed ciprofloxacin as the most effective by giving a disease control of 84.78, 97.78 and 80.43% when used as pre, simultaneous, and post inoculation sprays respectively, on cabbage heads. Other three antibiotics viz*.,* penicillin, tetracycline and oxytetracycline also gave good control of the disease. Factorial experiment aiming to study the combined effect of biocides and packing materials (polythene bags, net bags, gunny bag, and cardboard boxes) reveals that cabbage heads treated with ciprofloxacin and packed in net bags showed the lowest rot intensity of 4.5%, with lowest weight loss due to maceration after six days of storage, whereas cabbage heads packed in poly bags without any biocide treatment showed the highest rot intensity of 77.78% with the highest weight loss during the same course of time. These tested compounds and packing materials demonstrate the potential for management of post-harvest soft rot caused by *P. carotovorum* ssp. c*arotovorum* especially for regions lacking cold store facility during post-harvest and transit period.

Key words: *Pectobacterium carotovorum* ssp. C*arotovorum*, cabbage soft rot, post-harvest chemical control, packing material.

INTRODUCTION

Post-harvest bacterial soft rot causes greater total loss of product than any other bacterial disease (Agrios, 2007). Although accurate estimates of the losses are not available, these may amount annually to a total of $ 50 to 100 x 10^{6} on a world wide basis (Michel and Kelman, 1980). In cabbage (*Brassica oleracea* var *capitata* L.) and related crops, infected heads fail to throw flowering shoots followed by stump rot resulting in heavy losses in seed production. Vegetables coming from the field may already be infected even though they do not show visible symptoms at harvest. This latent infection may cause severe post-harvest damage because of high temperature and humidity. *Pectobacterium carotovorum* ssp. c*arotovorum* (formerly *Erwinia carotovora* ssp. *carotovora*) has been found to be the most common

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bacterial pathogen associated with the soft rot disease (Larka, 2004). This bacterium enters plant tissues primarily through wounds, often created by insect feeding or bruising at harvest or during post harvest handling. The conditions for the development of the disease become favorable with increase in temperature during the summer. The disease proliferates rapidly and within no time, results in rapid tissue breakdown and thus causes heavy damage especially in countries lacking cold storage and with poor transportation and handling system (Higashio and Yamada, 2004).

There are no effective chemical controls for *P. carotovorum* and other bacteria causing soft rot (James et al., 2009). Hot water treatment is recognized as a simple and easy technique to control pathogen infection and insect infestation and has already been put to practical use in many foreign countries (Gonzalez-Aguliar, 1999; Miura, 2001). However, in a study conducted by Higashio and Yamada (2004), no suppressive effect by dipping cabbage heads in hot water having temperature of 50°C was obtained. Energy costs associated with the process may also be prohibitory (Mills et al., 2006). Chlorination using sodium or calcium hypochlorite has been recommended (Galati et al., 2005). However, chlorine quickly loses effectiveness when soil, leaves or diseased tissue are present in the water; moreover, disinfectant activity of a chlorine solution increases at lower pH as greater the amount of hypochlorus acid becomes available for disinfection. However, vegetables and fruits are damaged by low pH chlorine solutions, moreover, chlorination acts as a preventive measure only and if there is already infection and decay or injury in the field, it does not help to limit spread of soft rot in storage (Anonymous, 1990). Although experiments investigating the effect of alum, silica gel and lime as post-harvest treatments to control soft rot in cabbage have shown decrease in the amount of infection (Napitupulu and Lubis, 1987, Sihombing, 1986), certain studies also reveal that salts are effective against various bacterial and fungal post-harvest infections in apples and potatoes (McGuire and Kelman, 1986; Conway et al., 1991). Other studies have shown that though many compounds of calcium proved effective, yet some of them showed a tendency to promote soft rot and the effect of calcium compounds application was due to the interception of bacteria at wounds rather than any physical function of calcium (Higashio and Yamada, 2004). Compounds like sodium metabisulphate, propyl paraben, alum, potassium sorbate, calcium propionate and copper sulphate pentahydrate showed that they inhibited *E. carotovora* ssp. *atroseptica* and *E. carotovora* ssp. *carotovora* (Mills et al., 2006) but an effective and promising control of the disease is still a challenge. Apart from chemical treatments, the effect of type of packing material on development of bacterial soft rot of vegetables in general has not received great attention.

An efficient packing material which considerably suppresses post harvest soft rot will be even more helpful in regions lacking cold store facility during post harvest and transit for a period until the produce reaches the consumer.

The present study aims to work out the *in vitro* effect of various antibiotics along with other bactericides on the soft rot causing bacteria *P. carotovorum* ssp. c*arotovorum* to test *in vitro* the effective ones as preventive and curative treatments against cabbage soft rot. Moreover, the effect of commonly used packing materials coupled with biocide treatments against soft rot disease in artificially infected cabbage heads with optimum epiphytotic conditions under storage was tested. Such a study was considered imperative in regions where the disease becomes highly destructive during the summer months.

MATERIALS AND METHODS

Studies were conducted in plant pathology laboratory of S.K. University of Agricultural Sciences and Technology of Kashmir. All the compounds used were obtained from the authorized suppliers approved by the university purchase committee. The strain of the pathogen used in the study was isolated from diseased tissue of cabbage tissue bearing soft rot lesions obtained from local wholesale and retail markets. The leaves were washed with sterile distilled water and several small diseased bits were excised from the leading edge. These bits were surface sterilized in 1:1000 mercuric chloride solution followed by a series of washings with sterile distilled water. These surface sterilized bits were macerated in sterile distilled water under aseptic conditions and kept undisturbed for two hours. A loopful from this suspension was streaked on at least 2 to 3 nutrient agar plates. The plates were incubated at 30±1°C for 48 h and observations on colony development were made. The isolated pathogen was characterized on the basis of pathogenicity on cabbage, morphological and biochemical characters. The identity of causal pathogen was also ascertained with the help of phytobacteriology division of Department of plant Pathology, Indian Agriculture Research Institute, New Delhi.

In vitro evaluation of chemicals against soft rot bacterium

The standard paper disc method of Thornberry (1950) was followed in this study. Nutrient agar was prepared and sterilized in usual manner in 250 ml flasks. The flasks were allowed to cool down up to 45°C and 1 ml of 48 h old bacterial broth culture was added aseptically to them and shaked properly. The medium thus seeded with the pathogen was immediately poured in 10 cm Petri dishes and allowed to solidify. Filter paper discs, 7 mm in diameter, were soaked aseptically for 5 min in aqueous solution of different chemicals viz*.,* ciprofloxacin, penicillin, tetracycline, oxy tetracycline, amoxicillin, striplin (streptomycin + tetracycline, 90:10), azithromycine, mercuric chloride, bleaching powder, sodium hypo chloride, and copper-oxy chloride, each used at three different concentrations. For each treatment, three Petri plates were used. In the centre of each Petri dish, one impregnated paper disc was placed. In case of control, paper discs soaked in sterile distilled water were used. The Petri plates were incubated for 72 h at 30°C. The efficacy of the various chemicals was assessed by measuring the zone of inhibition surrounding the filter paper disc after the incubation period.

Laboratory evaluation of chemicals against soft rot on cabbage heads.

Four biocides that proved effective against the pathogen during *in vitro* studies were selected for this experiment. The chemicals at a concentration of 200 µg mL^{-1} were applied at different times in three different experiments viz., 12 h prior to the inoculation of the pathogen, simultaneously with inoculation of the pathogen, and 12 h after inoculation of the pathogen. Freshly cut pre-weighed cabbage heads were surface sterilizes by dipping in a 0.1% of sodium hypochlorite for 30 s followed by serial washings with sterile water and then air dried under the hood of laminar air flow. Inoculum was generated from 48 h old culture of *P. carotovorum* ssp. c*arotovorum* grown in nutrient broth and the heads were thoroughly sprayed with the uniform cell suspension of the pathogen having a cell count of $2x10^9$ cfu/ml. In case of prior inoculation with pathogen, chemicals were sprayed 12 h after inoculation of pathogen. In case of simultaneous inoculation of pathogen and biocide, heads were inoculated with pathogen followed by pricking and immediately sprayed with test biocides. In the third case, at 12 h after with the biocides, the heads were sprayed with uniform suspension of the pathogen at the concentration afore reported. Cabbage heads in each case after inoculation with pathogen were followed by pin pricking with entomological pins mounted on a wooden stick. Cabbage heads inoculated with sterile distilled water followed by pin pricking served as control. Five cabbage heads constituted one replication and 3 replications were used for each treatment. The heads were put in sterile plastic bags, sealed and stored at 30±1°C.

Observations were recorded as soft rot incidence, severity and percent loss in weight due to the disease after 72 h of inoculation of pathogen. Incidence was calculated as percentage of heads showing symptoms and severity was calculated by the formula: {Sum of the score / (total heads observed x highest rating)} x 100. Disease severity was rated on 0 to 5 scale (0 = no maceration of the tissue, 1 = 1 to 20%, 2 = 21 to 40%, 3 = 41 to 60%, 4 = 61 to 80% and 5 = 81 to 100% of tissue maceration). Percent soft rot control was calculated using the formula: {(C-T)/C} x 100, where C= soft rot severity in control and T= soft rot severity in treatment. To calculate percent loss in weight due to disease, cabbage heads were weighed before incubation and again after removing decayed tissue after incubation. The final weight was subtracted from initial mass to give total amount of decayed tissue or loss in weight. Loss in weight due to drying was deduced with the help of un-inoculated controls.

Evaluation of chemicals and packaging material in combination for post harvest management of soft rot disease of cabbage

An experiment was carried out using pre-weighed surface sterilized cabbage heads to study the effect of the combined use of different packing materials (viz., polythene bags, net bags, gunny bag, and cardboard boxes) and in ciprofloxacin (200 ppm), alum (15% aluminium potassium sulphate) and lime. In case of ciprofloxacin and alum treatments, the chemicals were sprayed on the heads followed by spraying with the uniform suspension of the pathogen having a cell count of $2x10^9$ cfu/ml and pricking, while in case of lime treatment, the stalks of the heads were cut smoothly and were pressed on the powdered lime towards the butt end, followed by the spray of the pathogen suspension. All the treated heads were packed in the respective packing and stored at 30±1°C. In each case, cabbage heads without the inoculation of the pathogen were maintained to assess the weight loss due to storage. Observations were recorded on incidence and severity of soft rot after every 24 h for a period of 7 days and percent loss in weight due to the disease was calculated.

Statistical analysis

Experiments were carried in completely randomized design (CRD). Data collected was subjected to analysis of variance technique (ANOVA) and the least significant difference (LSD/C.D) at 5 % level of significance was calculated using statistical software STATISTICA-AG (Stat Soft USA) licensed to Faculty of Agriculture SKUAST-K, Wadura Campus Sopore, Kashmir, India.

RESULTS AND DISCUSSION

A total of eleven antibiotics and other biocides were tested *in vitro* for their ability to inhibit the growth of causal bacterium. All of the eleven chemicals tested against *P. carotovorum* ssp. c*arotovorum* viz., ciprofloxacin*,* penicillin, tetracycline, oxytetracycline, amoxicillin, stripling [streptomycin + tetracycline (90:10)], azithromycine, mercuric chloride, bleaching powder, sodium hypochlorite and copper oxychloride inhibited to varying extent the bacterial growth at all the three tested concentrations to varying extent and were considered effective. Ciprofloxacin proved significantly better than all other chemicals tested followed by penicillin. Others which followed in decreasing order of superiority were tetracycline, oxytetracycline and amoxicillin, respectively, followed by mercuric chloride, bleaching powder, azithromycine and striplin. Sodium hypochlorite and copper oxyxhloride gave only poor inhibition of the bacterium. No zone of inhibition was formed in case of control (Figure 1).

The study on effect of antibiotic treatment at a concentration of 200 µg ml^{-1} 12 h before inoculation of pathogen on cabbage heads (Figure 2) reveals that all the antibiotics tested for the control of post harvest soft rot disease in cabbage were effective over untreated control in reducing incidence, severity as well as per cent weight loss due to the disease (Table 1). Ciprofloxacin proved to be the most effective in reducing disease incidence followed by penicillin and tetracycline. Highest soft rot control of 84.78% was recorded in case of ciprofloxacin, followed by penicillin (78.26%), tetracycline (73.91%) and a comparatively lower control was recorded in case of oxytetracycline (67.38%) (Figure 2). The weight loss due to maceration of host tissue also showed a similar trend (Table 1).

Effect of antibiotic treatment at the time of inoculation with the pathogen on cabbage heads (Figure 3; Table 1) shows that all the treatments proved significantly superior over the control. Ciprofloxacin at 200 ppm was again most effective, giving least disease incidence (6.67) intensity (1.33) and percent weight loss of, and 0.51% followed by oxytetracycline, penicillin and tetracycline which did not differ significantly from each other but differed significantly from control. Ciprofloxacin controlled

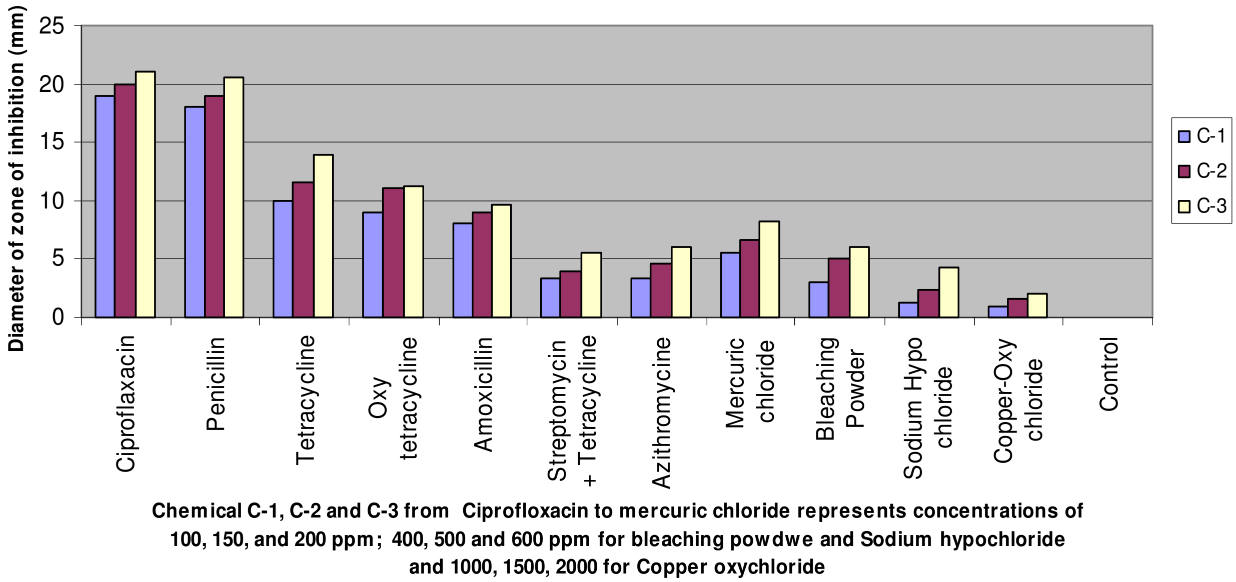

Figure 1. Effect of various chemicals on growth of *P. caratovorum* ssp. *caratovorum*.

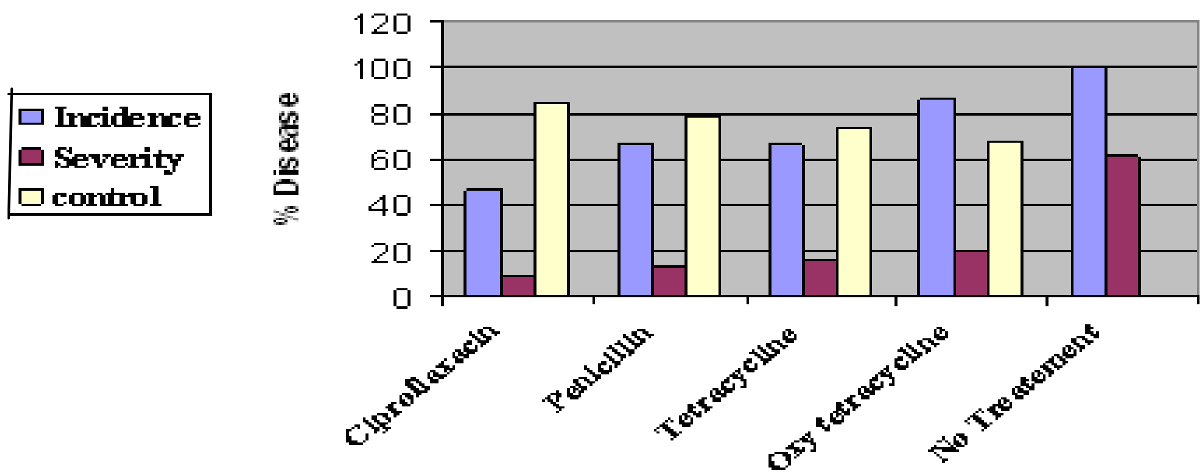

Figure 2. Effect of antibiotic treatment 12 h before inoculation of pathogen on cabbage heads stored at 30°C. Concentration of chemicals = 200 µg mL^{-1}.

Table 1. Effect of antibiotic treatment on per cent weight loss due to maceration caused by soft rot of cabbage.

Antibiotic / Time of application	Before 12 h pathogen	Simultaneously with pathogen	After 12 h of pathogen	Mean
Ciprofloxacin	2.55 (1.87)*	0.51 (1.22)	2.85 (1.94)	1.97 (1.68)
Penicillin	3.45 (2.09)	2.19 (1.78)	4.29 (2.29)	3.31 (2.05)
Tetracycline	4.08 (2.25)	2.58 (1.89)	4.05 (2.24)	3.57 (2.12)
Oxy tetracycline	5.07(2.46)	1.47 (1.55)	4.35 (2.30)	3.63 (2.11)
No treatment	29.42 (5.51)	29.42 (5.51)	29.42 (5.51)	29.42 (5.51)
Mean	8.91 (2.84)	7.23 (2.39)	8.99 (2.86)	8.38 (2.69)

C.D (p<0.05) (0.29)(0.35) (0.41). Treatments = 0.19; time of application = 0.15; treatments x time of application = 0.33; *figures in parenthesis are square root transformed values.

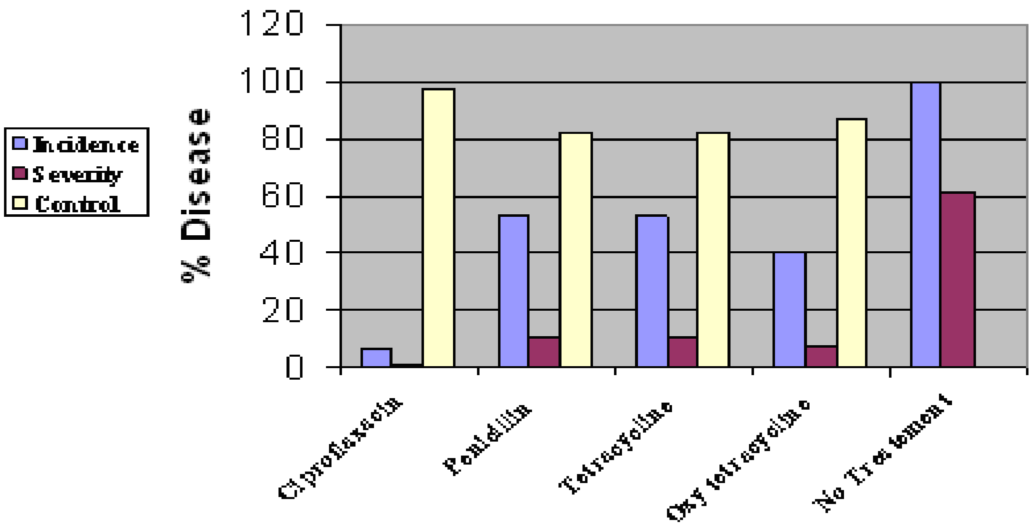

Figure 3. Effect of antibiotic treatment at the time of inoculation of pathogen on soft rot of cabbage heads stored at 30°C. Concentration of chemicals = 200 µg mL^{-1}.

the disease by 97.78%, followed by oxytetracycline (86.95%), penicillin (82.61%) and tetracycline (82.61%), respectively.

Similarly, upon the study of effect of antibiotic treatment 12 h after inoculation of pathogen, it is evident from the results presented in Figure 4 that all the antibiotics tested for the control of post harvest soft rot disease in cabbage were effective over untreated control in reducing incidence, intensity as well as percent weight loss due to disease (Table 1). Highest soft rot control of 80.43% was recorded in case of ciprofloxacin, followed by oxytetracycline (71.74%), penicillin (69.57%) and least was recorded in case of tetracycline (67.38%). The weight loss due to macerating effect of host tissue was least (2.85%) in heads treated with ciprofloxacin followed by tetracycline (4.05%), penicillin (4.29%) and oxytetracycline (4.35%) (Table 1). Data in Table 5 reveals that ciprofloxacin (200 ppm) spray was a superior treatment in all the three cases, that is, pre, simultaneous and post inoculation of all the treatments were significantly superior over control at all times of application.

The study shows that antibiotics can provide effective options for control of soft rot disease. The efficacy of ciprofloxacin which is a new fluoroquinolone antimicrobial agent is not well documented against *P. carotovorum* ssp. c*arotovorum*, but its efficacy is well established against Gram negative bacteria also belonging to enterobacteriaceae family (Anonymous, 2003). The present study confirms its efficacy against the enterobacteriaceae *P. carotovorum* ssp. c*arotovorum*. Grula et al. (1968) has reported inhibition of cell division of *Erwinia* sp. in low concentrations of penicillin. Mills et al. (2006) has reported seven penicillin binding proteins in *E. amylovora*. Tetracycline group of antibiotics which include tetracycline and oxytetracycline are inhibitors of protein synthesis and thus bactericidal (Anonymous, 1990). Best control of *E. carotovora* by streptomycin and tetracycline and partial control with copper compounds was also reported by Mills et al. (2006) and Mazzucchi and Swampa (1972). Present studies also confirm the bactericidal activity of such chemicals against *P. carotovorum* ssp. c*arotovorum.*

Results presented in Figures 5 and 6 reveals that lowest incidence of disease (11.12%) and severity of 2.20% (Figure 6) was recorded on cabbage heads in net bags treated with ciprofloxacin while cabbage heads packed in poly bags without any chemical treatment showed 100% disease incidence and 33.11% severity after 4 days of storage. Amongst packing materials, net bag packing was most efficient in reducing disease incidence followed by gunny bags and cardboard box packing, respectively. Cabbage heads packed in cardboard boxes and treated with ciprofloxacin showed a severity of 6.67%, which were at par with those packed in gunny bags and treated with lime (6.88%) (Figure 6). Disease incidence and severity in poly bag packing was highest. Similarly, among chemical treatments, ciprofloxacin proved most effective in reducing both incidence and severity followed by lime and alum, respectively.

Perusal of the data recorded after 6 days of storage (Figure 7) reveals that lowest incidence of disease (22.30%) was recorded on cabbage heads in net bags treated with ciprofloxacin while cabbage heads packed in polybags without any chemical treatment showed 100% disease incidence after 6 days of storage. Data reveals that amongst packing materials, net bag packing was most efficient in reducing the development of disease incidence followed by gunny bags and cardboard box packing, respectively. Similarly, among chemical

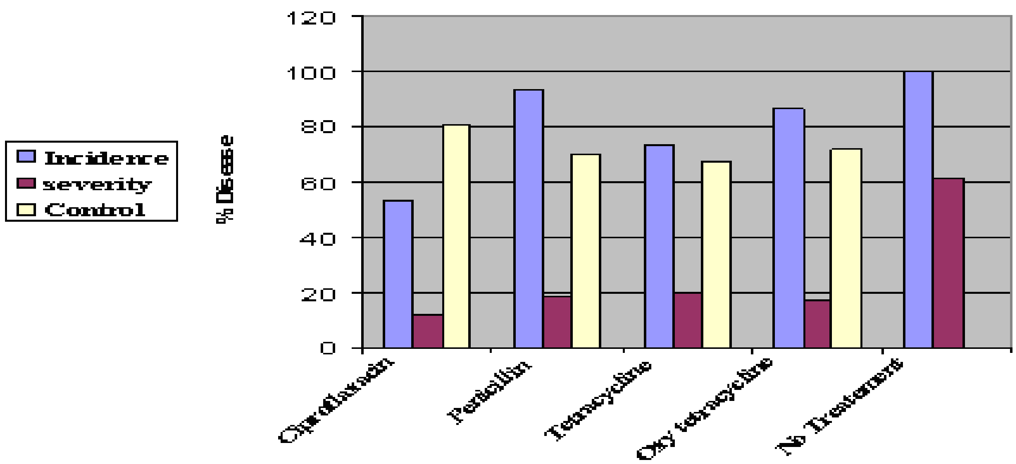

Figure 4. Effect of antibiotic treatment 12 h after the inoculation of pathogen on cabbage heads stored at 30°C. Concentration of chemicals = 200 µg mL^{-1}.

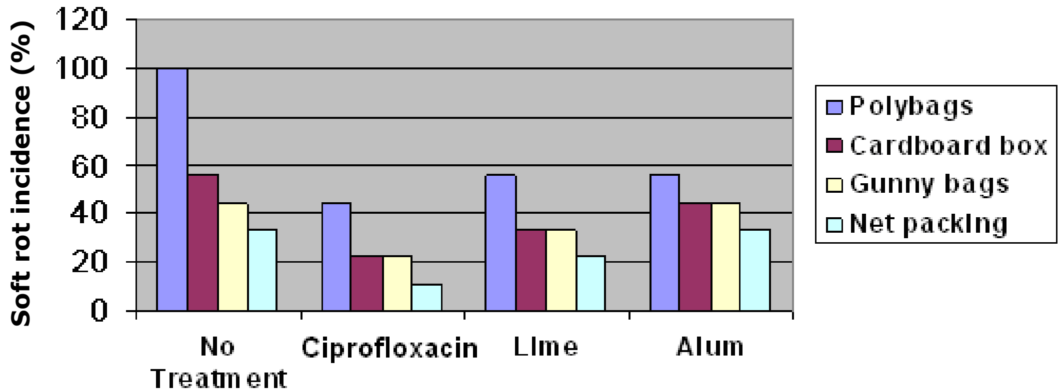

Figure 5. Effect of various packing materials and chemical treatments on incidence of soft rot of cabbage recorded after 4 days of inoculation.

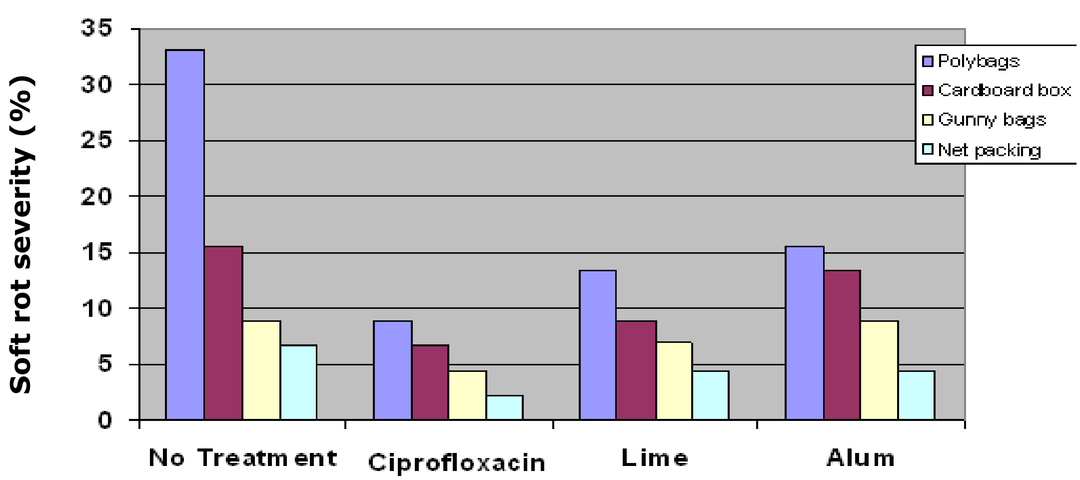

Figure 6. Effect of various packing materials and chemical treatments on severity of soft rot of cabbage recorded after 4 days of inoculation.

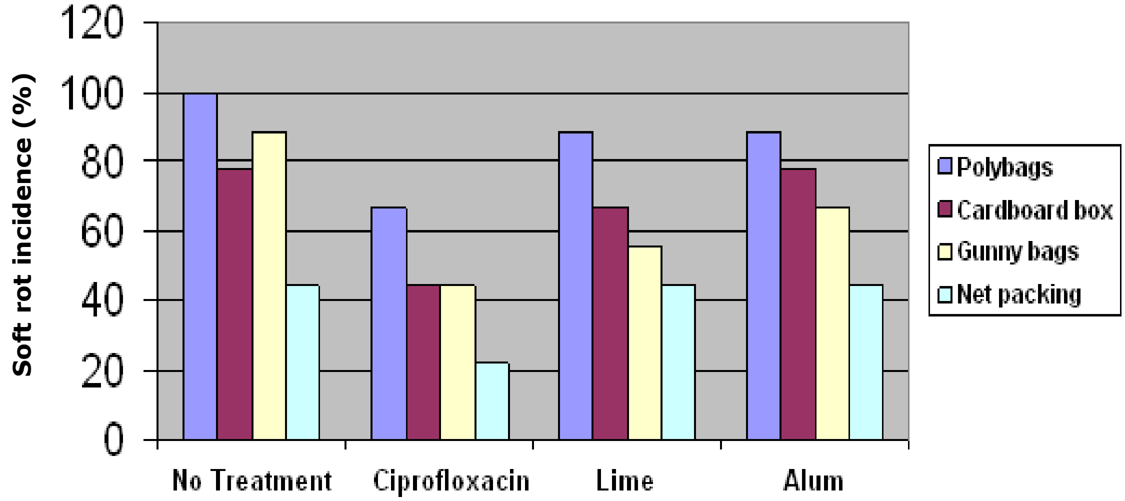

Figure 7. Effect of various packing materials and chemical treatments on incidence of soft rot of cabbage recorded 6 days after inoculation.

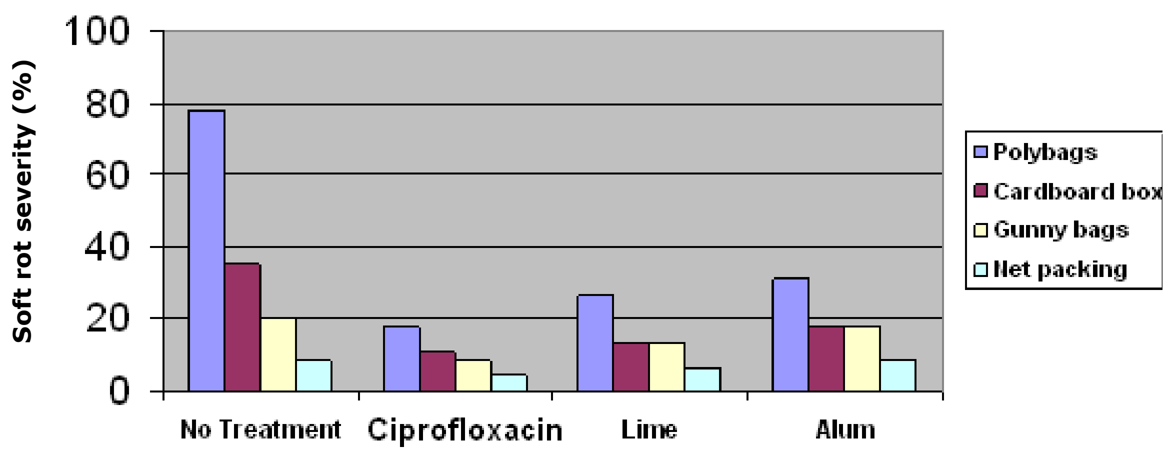

Figure 8. Effect of various packing materials and chemical treatments on severity of soft rot of cabbage recorded 6 days after inoculation.

treatments, ciprofloxacin proved most effective in reducing incidence followed by lime and alum, respectively.

Data on percent disease severity after 6 days (Figure 8) reveals that lowest severity of 4.50% was recorded in heads packed in net bags and treated with ciprofloxacin followed by 6.50% in heads packed in net bags and treated with lime. Heads stored in gunny bags and treated with ciprofloxacin showed severity at par with the heads stored in net bags and treated with alum (8.89%). The average effect indicates that net packing was most efficient with severity of 7.77% followed by gunny bag packing (14.99%). Among chemical treatments, ciprofloxacin was most efficient, showing an average severity of 10.56% followed by 15. 54% with lime, 18.89% with alum and 35.49% in heads receiving no treatment. The highest severity of 77.78% was recorded in heads stored in poly bags without chemical treatment.

The data presented in Table 2 on percent weight loss due to soft rot after 6 days indicates that minimum weight loss due to maceration (3.01%) was recorded in heads stored in net bags and treated with ciprofloxacin, which was followed by heads stored in gunny bags and treated with ciprofloxacin (5.70%). These were followed by heads stored in net bags and treated with lime which was statistically at par with those stored in net bags and treated with alum (6.70%). Highest weight loss of 69.50% was recorded in poly bags with no chemical treatment, followed by heads stored in cardboard box and receiving no treatment (29.33%). The average effect of packing material indicates that percent tissue loss due to maceration is lowest at 5.67% in net packing followed by

Table 2. Effect of various packing materials and chemical treatments on weight loss due to soft rot disease after 6 days of storage.

Packing material	Weight loss (%)				
	Chemicals				
	No treatment	Ciproflaxin	Lime	Alum	Mean
Polybags	69.50 (56.55)*	12.16 (20.23)	21.33 (27.46)	25.33 (30.21)	32.08 (33.61)
Cardboard box	29.33 (32.77)	6.03 (14.21)	9.26 (17.57)	12.16 (20.41)	14.04 (21.13)
Gunny bags	14.33 (22.23)	5.70 (13.77)	9.13 (17.69)	11.66 (19.50)	10.20 (18.15)
Net packing	6.36 (14.61)	3.01 (10.00)	6.63 (14.90)	6.70 (14.98)	5.67 (9.33)
Mean	29.88 (31.54)	6.75 (14.55)	11.60 (19.42)	14.09 (22.00)	15.58 (21.87)

C.D $_{(p\leq 0.05)}$. Chemicals = 1.61, packing = 1.61, chemicals x packing= 3.22; *figures in parenthesis are arcsine transformed values.

10.20% in gunny bag packing, 14.04% in cardboard packing and 32.08% in polybag packing. The average effect of chemicals indicates ciprofloxacin as highly effective chemical followed by butt end treatment of lime, and alum, as compared to heads stored in different packing materials without treatment.

The study to work out ideal packing material in combination with chemicals that inhibit post harvest soft rot in cabbage revealed that type of packing material played a very crucial role in development of the disease. Highest disease incidence, intensity and percent tissue loss due to maceration was observed in cabbage heads stored in poly bags without any chemical treatment. Moreover, a severity of 33.11% after 4 days of storage in polybag packing which increased to 77.78% after 6 days of storage clearly indicates the destructive nature of soft rot and its rapid development under favorable conditions of temperature and humidity. Although the heads receiving chemical treatments and stored in polybags showed considerable reduction of the disease, but despite treatments, the disease was comparatively higher than in case of other three packing materials. In cardboard boxes, disease was much less than that of poly bags. Among the cabbage heads stored in cardboard boxes and receiving different chemical treatments, the weight loss due to maceration was lowest at 6.03% in those treated with ciprofloxacin. Gunny bag packing was still better then poly bag as well as card board box packing, which results in less disease incidence, severity and percent weight loss. The rate of progress of disease was also less in gunny bags as compared to polybags and card board boxes. Again, in case of gunny bag packing, lowest disease was recorded in heads treated with ciprofloxacin and highest in heads receiving no chemical treatment. The most efficient packing material showing least disease incidence, severity, as well as tissue maceration was observed to be net bag packing and the most efficient combination of chemical treatment and packing material were cabbage heads treated with ciprofloxacin and packed in net bags. A lowest of 3.01% tissue loss due to maceration was recorded in heads packed in net bags and receiving ciprofloxacin treatment after six days of storage duration. Net bags besides preventing disease also considerably slowed down disease development upon 6 days of storage as compared to other packing materials.

Although there are no previous reports regarding use of packing materials to reduce post harvest soft rot losses, however, different packing materials play a vital role in maintaining different degrees of humidity and temperature in packages during storage and transit. High humidity in combination with favorable temperature is congenial for development of soft rot. Our studies is therefore supported by previous studies of Bhattacharya and Mukarjee (1986) and Raju et al. (2008), who as a result of their studies concluded that increased relative humidity (RH) enhanced soft rot in storage. Walker (1998) also reported dehydration of rot tissue and reduction of advancement of soft rot in dry atmosphere, which supports our findings that less humidity in net bags results in less post harvest soft rot.

Efficacy of chemicals like alum, against soft rot disease has previously been reported by several workers (Mills et al., 2006). Higashio and Yamada (2004) and Tsuyama (1961) have reported butt end treatment of cabbage heads with lime effective to prevent post harvest bacterial soft rot by interception of bacteria at wounds, received during harvesting of cabbage heads. These findings are in conformity with our studies. Although, during present investigations, a better chemical treatment (ciprofloxacin in combination with net packing) was found highly effective in reducing post harvest bacterial soft rot in cabbage up to 6 days of storage. Though antibiotics have already been used in various plant diseases control strategies, however, more studies are required before use of such antibiotics in cabbage or other food to rule out running of any possible risk of development of antibiotic resistant bacterial species and to estimate any possible health hazard related to such control strategies; besides, any such disease control strategies involving chemicals if used, should not be considered the ultimate, and research should continue to look for safer alternatives.

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