African Journal of Microbiology Research Vol. 4 (11) pp. 1100-1104, 4 June, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 © 2010 Academic Journals

Full Length Research Paper

Antifungal activities of neem (*Azadirachta indica*) seed kernel extracts on postharvest diseases in fruits

Jingfa Wang, Jian Li, Jiankang Cao and Weibo Jiang*

College of Food Science and Nutritional Engineering, China Agricultural University, P.O. Box 608, Qinghua Donglu No. 17, Beijing, 100083, China.

Accepted 17th May, 2010

To learn the antifungal effects of neem seed kernel extract (NE) on the post harvest diseases, pathogens of *Monilinia fructicola, Penicillium expansum, Trichothecium roseum* and *Alternaria alternate* isolated from the infected fruit were treated with NE *in vitro*. Results showed that growth of the four pathogens could be significantly ($P < 0.05$) reduced by NE. The diseases in fruit of plum (*Prunus salicina*) or Yali pear (*Pyrus bertschneideri*) inoculated with the pathogens could be prevented remarkably by treated fruit with NE.

Key words: Neem seed kernel extracts, antifungal, fruits, post harvest, pathogens.

INTRODUCTION

Considerable post harvest losses of fruit are brought about by decay caused by fungal plant pathogens and hence inhibiting the growth of fungi has great significance in post harvest fruit, but in recent years, the use of synthetic fungicides has increased consumer concern and their use is becoming more restrictive due to carcinogenic effects, residual toxicity problems, environmental pollution, occurrence of microbial resistance and high inputs (Diánez et al., 2002; Marín et al., 2003; Rial-Otero et al., 2005).

Recently, some higher plant products have attracted the attention of microbiologists to search for some phytochemicals for their exploitation as anti-microbials, Such plant products would be biodegradable and safe to human health (Kumar et al., 2008).

Yali pear (*Pyrus bertschneideri*) and plum (Prunus salicina) are typical kernel fruit and stone fruit planting in China, post harvest decay of them often caused by *Penicillium expansum, Trichothecium roseum*, Alt*ernaria alternate* or *Monilinia fructicola*. There are many ways to controlling decay of pear and plum, but using fungicides still is one of most important way. Application of post harvest fungicides to protect pear or plum against fungi attack has been studying for many years (Sugar et al., 2008; Svirceva et al., 2007), especially phytochemicals are attracting many researchers' efforts.

Neem (*Azadirachta indica Juss*), a large tree of India, has been used for centuries in Asia as insecticides, fungicides, anticonceptionals in popular medicine almost every part of this tree seeds, leaves, roots, bark, trunk and branches has multiple uses (Chaturvedi et al., 2003) and has been recommended to plant in African and Asia by many international organizations.

Some extracts from neem plant have been shown to be toxic to fungal pathogens, such as *Poria monticolad* infecting wood (Dhyani et al., 2004), *Aspergillus flavus* from soybean seeds (Krishnamurthy et al., 2008), *Pyricularia oryzae* infecting rice plant in field and the harvested rice (Amadioha, 2000). Therefore, it could be expected that the chemicals in need might also be effective against fungal pathogens in harvested fruits. So far, little information is available whether neem extract can be used in controlling fruit decay caused by plant pathogens during storage.

The purpose of this paper is to test the possibility of using extract from neem seed kernel to inhibit post harvest disease of fruit. Our results showed that the neem extract could effectively inhibit growth of fungal pathogens in post harvested fruit.

MATERIALS AND METHODS

Plant materials

Neem seed kernel was purchased from Guangming Neem Industry

*Corresponding author. E-mail: jwb@cau.edu.cn. Tel: 86-01-62736565. Fax: 86-01-62736565.

Table 1. Growth inhibition determined by agar diffusion method.

Pathogen	Colony diameter (mm)		Index of growth inhibition (%)
	Control	100 (mg NE/ml)	
M. fructicola	28.3 ± 0.6a	9.7 ± 0.6b	65.7
P. expansum	8.8 ± 0.3c	5.8 ± 0.3d	34.1
T. roseum	27.8 ± 1.1a	9.2 ± 1.0b	66.9
A. alternata	27.0 ± 1.0a	18.7 ± 0.6e	30.7

NE is the abbreviation of neem seed kernel extracts. All the fungi in petri dishes were incubated in dark at 27 ± 2℃ for 72 h. The data was analyzed by one-way analysis of variance (ANOVA), and expressed as mean value of 3 replicates± S.E. Means in the same line followed by different letters are significantly different ($P < 0.05$).

Development Co., LTD, China.

Plum (*Prunus salicina*) was purched from a local market in Beijing and Yali pear (*Pyrus bertschneiden*) was harvested from a local orchard in Hebei Province, China.

Neem extract

200 g neem seed kernel was ground into fine powder (less than 20 mesh) using a stainless-steel grinder, then extracted with 400 ml 100% ethanol for 20 min in the ultrasound bath. The filtered extract was concentrated by a rotary evaporator and finally 52 g sample of the extract (NE) was left for subsequent experiments.

Pathogens

The pathogens, including *M. fructicola*, *P. expansum*, *T. roseum*, *A. alternata* were isolated from infected fruit, then preserved in our lab and activated on potato dextrose agar (PDA) before using them.

In vitro assessment the antifungal activity of the neem extract

The assessment of antifungal activity of NE *in vitro* was conducted according to Rios et al. (1988). The NE was dissolved in sterilized PDA at 45℃ (final concentration was 100 mg NE per ml PDA), then poured it into 9 cm diameter glass Petri dishes (20 ml per dish). One 6 mm disc of the fungal species was cut from 1-week-old cultures on the PDA plates and then was placed on the centre of the dish (one disc per dish). For analysis of *P. expansum*, 6 mm disc was excavated from the PDA in center of the dish (one hole per dish) and a 50 µl conidial suspension of the *P. expansum* (10^6 conidia per ml) was filled into the hole and then incubated in dark at 27 ± 2℃ for 72 h. The relative growth inhibition of treatment compared to negative control was calculated as percentage mycelia growth inhibition according to Pandey et al. (1982). Index of growth inhibition (IGI) of the treatment on pathogen = (dc-dt)/dc × 100, where dc = average growth diameter of the control colony, dt = average growth diameter of the treated colony. Three Petri dishes per treatment, three replicates.

Inoculation and disease evaluation

The antifungal activity of NE *in vivo* in the fruit was assessed according to method of Liu et al. (2005), Cao et al. (2006) and Zhu et al. (2008) with slight modifications. Conidial suspension of the pathogen was prepared by flooding the 7-day-old culture dishes incubated at 27℃ with sterile-distilled water containing 0.01% Tween-80 and adjusted to 10^6 conidia per ml. Both the NE treated and control fruit (10 mature fruits per treatment, three replicates) were surface-sterilized with 70% ethanol and wounded with a sterilized nail at 2 points (2 mm deep 5 mm wide) on the equator of each fruit. 20 µl 50 mg/ml, 100 mg/ml and 200 mg /ml NE or sterile distilled water (control) was pipetted into each wound site and then 15 µl of the conidial suspension was injected into each of the wounded sites. Thereafter, the fruit were kept in plastic containers and incubated at 25℃, 95 - 100% RH. Lesion diameter on the fruit was recorded at the indicated times.

Data analysis

Statistical analysis of data was conducted with SPSS 12.0 program (SPSS Inc., Chicago, IL, USA) for Windows. The data of colony diameter in dish or lesion diameter on the fruit were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed using the least significant difference method (LSD test). P values of < 0.05 were considered as statistically significant.

RESULTS

In vitro assessment of NE effect

Growth of the pathogens was significantly ($P < 0.05$) inhibited by the NE. As shown in Table 1, the index of growth inhibition respectively was 65.7% for the *M. fructicola*, 34.1% for the *P. expansum*, 66.9% for the *T. roseum* and 30.7% for the *A. alternate*, respectively, after incubated at 27℃ for 72 h (Table 1).

Effect of NE on plum fruit inoculated with *M. Fructicola*

The infected lesions in plum fruit were clearly observed at 48 h after the inoculation. As shown in Figure 1, the lesion diameters (ID) in the plum fruit were significantly reduced by treated fruit with 50 mg/ml NE or higher concentrations of NE. The ID in the fruit treated with 50 mg/ml NE, 100 mg/ml NE or 200 mg/ml NE was 11.1, 20.9 and 30.6% lower than that in control fruit, respectively, at 48 h after the inoculation (Figure 1).

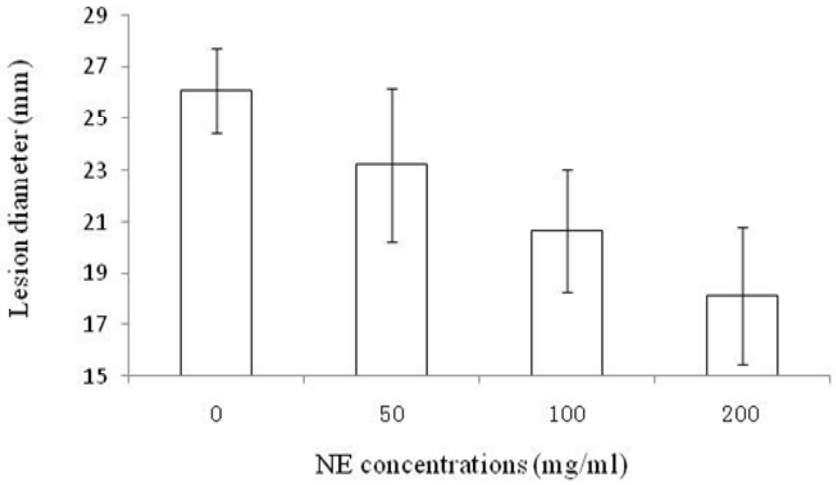

Figure 1. Effect of the NE on *M. fructicola* in plum fruit. The plums were wounded and treated with water control 50, 100 and 200 mg/ml NE and *M. fructicola*, then incubated at 25℃, 95 – 100% RH. The lesion diameters on each fruit were recorded in 48 h after the inoculation.

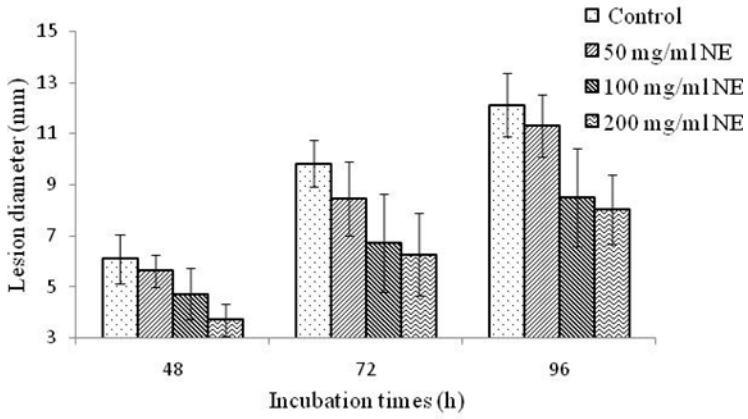

Figure 2. Effect of the NE on *A. alternata* in Yali pear fruit. The pears were wounded and treated with water control 50, 100 and 200 mg/ml NE and *A. alternata*, then incubated at 25℃, 95 – 100% RH. The lesion diameters on each pear were recorded after 48, 72 and 96 h.

Effect of NE on inoculated Yali pear fruit

Growth of the pathogenic fungi in the inoculated fruit of Yali pear was inhibited to a certain extent by the NE treatments. As shown in Figure 2, the lesion diameter (ID) on the fruit treated with 100 mg/ml NE or 200 mg/ml NE were only 83.7 or 58.5% of that on the control fruit after the inoculation with *A. alternata* for 96 h at 25℃ (Figure 2).

Similar effects of the NE treatment were also observed on the fruit inoculated with *T. roseum* or *P. expansum*. The ID on the fruits treated with 100 mg/ml NE or 200 mg/ml NE and inoculated with *T. roseum* was only 83.6 or 79.7% of that on the control fruit after the inoculation for 96 h (Figure 3).

The ID on the fruits treated with 50, 100 or 200 mg/ml NE and *P. expansum* was 74.2, 67.2 or 63.1%, respectively, less than that on the control fruits after the inoculation for 96 h (Figure 4). Whatever pathogens were inoculated, the ID on the fruits treated with 200 mg/ml NE was significantly ($P < 0.05$) less than that on the control fruit in our experiment (Figures 3 and 4).

DISCUSSION

Our results indicated that neem seed kernel extracts NE can effectively inhibit some post harvest fruit pathogens

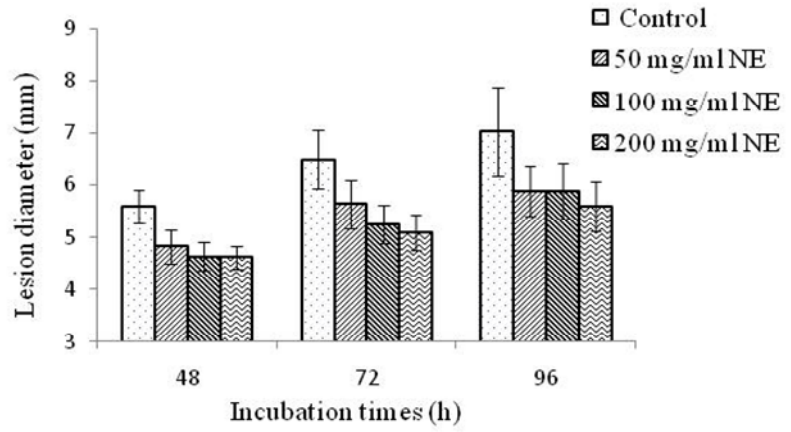

Figure 3. Effect of NE on *T. roseum* in Yali pear fruit. The pears were wounded and treated with water (control), 50, 100 and 200 mg/ml NE and *T. roseum*, then incubated at 25℃, 95 – 100% RH. The lesion diameters on each pear were recorded after 48, 72 and 96 h.

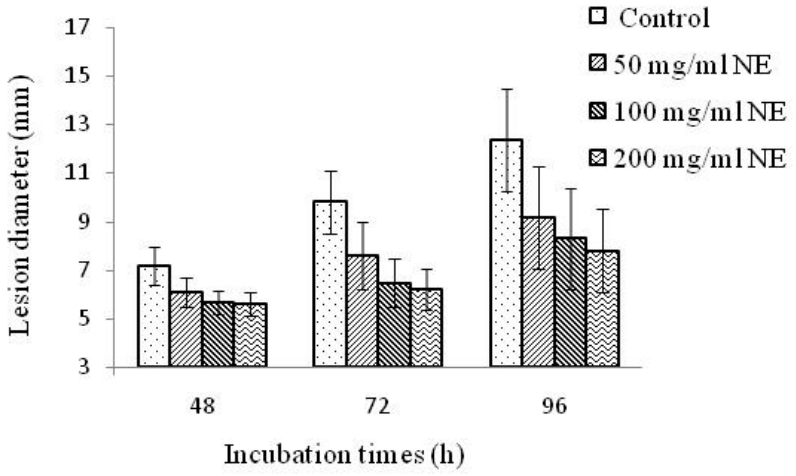

Figure 4. Effect of NE on *P. expansum* in Yali pear fruit. The pears were wounded and treated with water (control), 50, 100 and 200 mg/ml NE and *P. expansum*, then incubated at 25℃, 95 – 100% RH. The lesion diameters on each pear were recorded after 48, 72 and 96 h.

in *vitro* and *in vivo*, but it should be noted that this study has examined only crude NE with simple extracting method. Sairam et al. (2000) has reported that some components for their antimicrobial activity, NIM-76, a spermicidal fraction from neem oil, was investigated for its antimicrobial action against certain bacteria, fungi and polio virus, as compared to whole neem oil, The NIM-76 preparation showed stronger antimicrobial activity than the whole neem oil. As the crude NE what we used may contain many kinds of components, there may exists the possibility to further isolating the crude NE to decrease the using volume against pathogens.

Paul and Sharma (2002) reported that germination of spores of *Drechslera graminea* effectively could not be inhibited by neem extract. Our results showed that, although the treatments with neem extract did not remarkably inhibit germination of the pathogenic spores, they could postpone formation of the spores. No spore of *A. alternata* was observed when the fungi being incubated with PDB containing 20 mg/ml decreased NE (200 g neem kernel was ground and decreased with petroleum before extraction, then 13.6 g decreased NE was got) for 7days, though the fungal spores could be observed in the control in 2 or 3 days after the incubation.

There are many kinds of nature products have been studied on plant protection, some of them are considered

have the ability of inducing plant defence reactions (Ghaouth et al., 1997; Sticher et al., 1997; Meir et al., 1998; Wang et al., 2009).

The lesion development of Yali pear and plum may be mainly affected by directly inhibiting the growth of fungi, Paul and Sharma (2002) think that neem extract maybe can induce plant defense reactions and be useful in management of leaf stripe disease of barley and Cao et al. (2006) proved that disease resistance in harvested Yali fruit can be affected by salicylic acid treatment on the trees in field during fruit growth and development, the NE has the ability of inhibiting the growth of fungi through inducing plant defense reactions and postponing formation of the spores in fruit of Yali pear and plum, we think it should be more significant to do it in the field.

Extracts of neem are being extensively used to control pests (Akhtar et al., 1997; Michereff et al., 2008). Several pesticides whose main components extracted from neem are using on protecting garden plants in China now. When these pesticides are used in field, can it reduce the post harvest losses of Yali pear and plum caused by fungal pathogens? If it can do so, what is the mechanism? Further studies are necessary to learn more on the functions of neem components.

ACKNOWLEDGEMENT

This research was supported by the 11th Five-Year Key Technologies R and D Program of China (2006BAD22B03).

REFERENCES

Akhtar M, Mahmood I (1997). Control of root-knot nematode *Meloidogyne incognita* in tomato plans by seed coating with suneem and neem oil. J. Pestic. Sci., 22: 37-38.

Amadioha AC (2000). Controlling rice blast in vitro and in vivo with extracts of *Azadirachta indica*. Crop Prot., 5: 287-290.

Cao J, Zeng K, Jiang W (2006). Enhancement of postharvest disease resistance in YaLi pear (*Pyrus bretschneideri*) fruit by salicylic acid sprays on the trees during fruit growth. Eur. J. Plant Pathol., 114: 363-370.

Chaturvedi R, Razdan MK, Bhojwani SS (2003). Production of haploids of neem (*Azadirachta indica A. juss.*) by anther culture. Plant Cell Rep., 21: 531-537.

Dhyani S, Tripathi S, Inder D (2004). Preliminary screening of neem (*Azadirachta indica*) leaf extractives against Poria monticolad-a wood destroying fungus. J. Indian Acad. Wood Sci., 1-2: 103-112.

Diánez F, Santos M, Blanco R, Tello JC (2002). Fungicide resistance in *Botrytis cinerea* isolate from strawberry crops in Huelva (southwestern Spain). Phytoparasitica, 30: 529-534.

El Ghaouth A, Arul J, Wilson C, Behamou N (1997). Biochemical and cytochemical aspects of the interaction of chitosan and *Botrytis cinerea* in bell pepper fruit. Postharvest Biol. Tec., 12: 183-194.

Krishnamurthy YL, Shashikala J, Naik BS (2008). Antifungal potential of some natural products against *Aspergillus flavus* in soybean seeds during storage. J. Stored Prod. Res., 44: 305-309.

Kumar A, Shukla R, Singh P, Prasad CS, Dubey NK (2008). Assessment of Thymus vulgaris L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. Innov. Food Sci. Emerg., 4: 575-580.

Liu H, Jiang W, Bi Y, Luo Y (2005). Postharvest BTH treatment induces resistance of peach(Prunus persica L. cv. Jiubao)fruit to infection by *Penicillium expansum* and enhances activity of fruit defense mechanisms. Postharvest Biol. Tec., 35: 263-269.

Marín A, Oliva J, García C, Navarro S, Barba A (2003). Dissipation rates of cyprodinil and fludioxinil in lettuce and table grape in the filed and under cold storage conditions. J. Agr. Food Chem., 51: 4708-4711.

Meir S, Droby S, Davidson H, Alsevia S, Cohen L, Horev B, Pilosophadas S (1998). Suppression of Botrytis rot in cut rose flowers by postharvest application of methyl jasmonate. Postharvest Biol. Tec., 13: 235-243.

Michereff M, Torres JB, Andrade LNT, Nunes MUC (2008). Effect of some biorational insecticides on Spodoptera eridania in organic cabbage. Pest Manag. Sci., 64: 761-767.

Pandey DK, Tripathi NN, Tripathi RD, Dixit SN (1982). Fungitoxic and phytotoxic properties of essential oil of Hyptis suaveolens. J. Plant Dis. Protect., 89: 344-349.

Paul PK, Sharma PD (2002). Azadirachta indica leaf extract induces resistance in barley against leaf stripe disease. Physiol. Mol. Plant Pathol., 61: 3-13.

Rial-Otero R, Arias-Estévez M, López-Periago E, Cancho-Grande B, Simal-Gándara J (2005). Variation in concentrations of the fungicides tebuconazole and dichlofluanid following successive applications to greenhouse-grown lettuces. J. Agr. Food Chem., 53: 4471-4475.

Rios JL, Recio MC, Villar A (1988). Screening methods for natural products with antimicrobial activity: a review of the literature. J. Ethnopharmacol., 23 : 127-149.

Sairam M, Llavazhagan G, Shama SK, Dhanraj SA, Suresh B, Parida MM, Jana AM, Devendra K, Selvamurthy W (2000). Anti-microbial activity of a new vaginal contraceptive NIM-76 from neem oil(Azadirachta indica). J. Ethnopharmacol., 71: 377-382.

Sticher L, Mauch-Mani B, Métraux JP (1997). Systemic acquired resistance. Annu. Rev. Phytopathol., 35: 235-270.

Sugar D, Basile SR (2008). Timing and sequence of postharvest fungicide and biocontrol agent applications for control of pear decay. Postharvest Biol. Tec., 49: 107-112.

Svirceva AM, Smith RJ, Zhou T, Hernadez M, Liu W, Chu CL (2007). Effects of thymol fumigation on survival and ultrastracture of Monilinia fructicola. Postharvest Biol. Tec., 45: 228-233.

Wang F, Feng G, Chen K (2009). Defense responses of harvested tomato fruit to burdock fructooligosaccharide, a novel potential elicitor. Postharvest Biol. Tec., 1: 110-116.

Zhu X, Cao J, Wang Q, Jiang W (2008). Postharvest Infiltration of BTH Reduces Infection of Mango Fruits (Mangifera indica L. cv. Tainong) by Colletotrichum gloeosporioides and Enhances Resistance Inducing Compounds. J. Phytopathol., 156: 68-74.