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Medicinal Plant Extracts and Protein Kinase C Inhibitor Suppress Zoosporogenesis and Impair Motility of *Phytophthora capsici* Zoospores

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Abstract

Ansary M.W.R., Haque E., West H.M., Rahman M.M., Akanda A.M., Wang Y., Islam M.T. (2016): **Medicinal plant extracts and protein kinase C inhibitor suppress zoosporogenesis and impair motility of** *Phytophthora capsici* **zoospores**. Plant Protect. Sci., 52: 113–122.

The effects of water and acetone extracts from 100 medicinal plants growing in Bangladesh, along with a selective inhibitor of protein kinase C (PKC), chelerythrine chloride, were tested on zoosporogenesis (release of zoospores from the sporangia) and motility of *Phytophthora capsici* zoospores. Among 10 active crude acetone extracts, those from *Psidium guajava* and *Nigella sativa* (100 µg/ml) suppressed zoosporogenesis relative to the control (100% zoospore release) to 60 and 40% released, respectively and inhibited motility of 100% of the zoospores within 60 min of treatment. Chelerythrine chloride also suppressed zoosporogenesis (30% released) at 0.1 µg/ml and inhibited motility of 100% zoospores at 0.2 µg/ml within 60 minutes. Among water extracts of 100 medicinal plants, 56 impaired motility of zoospores in a dose- and time-dependent manner. Diluted (20-fold) water extracts of 10 plants including *Ocinum gratissimum, Terminalia bohera*, and *Duranta plumeri* inhibited motility and subsequently caused lysis of zoospores. As the inhibition of zoosporogenesis and zoospore motility limit the possibility of infection by the peronosporomycete phytopathogen, the inhibitory crude extracts of medicinal plants identified in this study should have great potential for practical use as biopesticides against *P. capsici*.

Keywords: secondary metabolits; biopesticides; chelerythrine chloride; bioassay; zoospore motility; *Nigella sativa*

Phytophthora capsici is a serious pathogen causing Phytophthora blight, root or crown rot in chilli, cucumber and some other plants belonging to the Solananceae and Cucurbitaceae families worldwide (Erwin & Ribeiro 1996; Ristaino & Johnston 1999; McGrath 2004). The estimated worldwide annual crop loss due to *Phytophthora* is several billions of dollars (McGrath 2004). This fungus-like organism belongs to the class Peronosporomycetes within the kingdom of Straminipila and relatives include golden-brown algae, diatoms, and brown algae (Dick 2001). One of the unique features of peronosporomycete phytopathogens is that they asexually produce motile zoospores, which are propelled by two dissimilar flagella (heterokont) (Islam & Tahara 2001). These motile zoospores are an important means of distribution and are often

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the key infectious stage of the pathogen's life cycle (Deacon & Donaldson 1993; Islam & Tahara 2001; Judelson & Blanco 2005). Our knowledge of the biology of *Phytophthora* is still limited. Due to unique cell biology, zoospores are insensitive to most fungicides; some copper-containing substances are effective in controlling *Phytophthora* diseases, but these have detrimental effects on public health, environment, non-target organisms, and development of resistance (Kagale *et al*. 2004; Rai & Carpinalla 2006; Bahraminejad *et al*. 2012). To develop a biorational control measure against *Phytophthora* and to find novel targets, new approaches are needed.

Extracts of some traditional medicinal plants (non-host) affect the motility and viability of the zoosporic pathogens, *Aphanomyces cochlioides* and *Plasmopara viticola* (Begum *et al*. 2002; Islam *et al*. 2002; 2003; Islam 2008, 2013; Islam & Hossain 2013). Inhibitory plant extracts have been shown to contain bioactive compounds such as nicotinamide, polyflavonoid tannins, 2-methoxy-5-methyl-6-methoxymethyl-*p*-benzoquinone, and camphor (Islam *et al*. 2002; Kordali *et al*. 2005; Chen *et al*. 2012). Some of these natural compounds also impaired zoospore motility and caused lysis of zoosporic pathogens. In addition, protein kinase C (PKC) inhibitors impairs zoospore motility and suppresses pathogenesis in grapevine by *Plasmopara viticola* (Islam *et al*. 2011). However, studies concerning screening of non-host plant extracts against *P. capsici* are scant. Bangladesh has a rich diversity of traditional medicinal plants and *P. capsici* is one of the major phytopathogens in the country (Hossain & Friedt 2010). To date, no study has been conducted to screen Bangladeshi medicinal plant extracts or PKC on zoosporogenesis, motility, and viability of zoospores of any species of phytopathogenic *Phytophthora*. Chelerythrine is a benzophenanthridine alkaloid present in the plant species *Chelidonium majus* and in several species of *Zanthoxylum*. Chelerythrine is a selective inhibitor of PKC and antimicrobial against *Staphylococcus aureus* and other human pathogens (Tavares *et al*. 2014). Therefore, a survey of inhibitory extracts from non-host plants which are capable of suppressing zoosporogenesis in *P. capsici* and of impairing zoospore motility would be important for the development of an eco-friendly plant-based biopesticide. Therefore, the objective of this study was to evaluate the effects of plant extracts and a PKC inhibitor, chelerythrine chloride on zoosporogenesis, motility, and lysis of *P. capsici* zoospores.

MATERIAL AND METHODS

Materials and chemicals. Samples (leaf, stem, seeds or roots) of 100 medicinal plants were collected from Dhaka, Gazipur, and Rajshahi districts of Bangladesh. *Phytophthora capsici* was obtained from Nanjing Agricultural University, China. All chemicals used including chelerythrine chloride and acetone were analytical or of HPLC grade.

Plant extraction and preparation for bioassay. Five gramms of each surface sterilised (with 70% ethanol) plant sample (stem/leaf/bark/flower) were ground and then suspended into 5 ml distilled water in a screw-capped glass vial. After one day, the supernatant was used for primary screening. For large-scale extractions, 30 g of each dry plant sample were finely ground using an electrical grinder. According to the bulkiness of plant materials, acetone (80 ml) was added to each sample in a volumetric flask and allowed to stand for 7 days at room temperature, after which the supernatant was concentrated *in vacuo* (Islam *et al*. 2002, 2004). Stock solutions of the crude acetone extracts were prepared in dimethyl sulfoxide solution (DMSO). The concentration of DMSO never exceeded 1%, which does not affect zoospore motility.

***Inhibitory activity test against** P. capsici*. *P. capsici* was maintained on 10 ml of sterile carrot piece agar in 90-mm Petri dishes at 25°C in the dark. Colonies were regularly sub-cultured by introducing a plug of hyphal tips onto fresh agar and incubating. To induce sporangia formation, agar cultures from 2-week-old Petri dishes were cut into pieces, covered with sterile distilled water, and kept in Petri dishes at 25°C in the dark. To induce zoospore release, cultures with sporangia were placed at 4°C for 30 min, and then incubated at room temperature for another 30 minutes. The sporangia and release of zoospores were checked under a compound microscope. Zoospores remained motile for 10–12 h in sterile water and were used for the bioassays. Inhibitory activity of crude acetone extracts on zoosporogenesis was tested as described previously by Islam *et al*. (2011) and motility behaviour and viability of zoospores were tested by the "homogeneous solution method" (Islam *et al*. 2004). Briefly, zoospore suspensions (360 µl) were added to clean Petri dishes and each suspension was tested individually against different concentration of water extract or crude acetone extract (in 1% DMSO) derived from the medicinal plant samples. The behaviour of zoospores was ob-

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served under a compound microscope and recorded relative to that of control suspensions (zoospore suspensions in sterile distilled water or with 2% DMSO). Quantification of time-course changes of motility and lysis of zoospores was carried out as described earlier (Islam *et al*. 2002). Data are expressed as percentage of zoospores released (calculated as a proportion of the control value, 100%), percentage inhibition of motility (of the tested zoospores), and percentage of lysis and are shown as mean values ± standard error of the mean. The experimental design was completely randomized with three replicates per treatment and each bioassay was repeated twice.

RESULTS

***Plant extracts suppress release of** P. capsici **zoospores (zoosporogenesis)**.* To determine whether plant extracts suppress release of *P. capsici* zoospores from the sporangia, appropriate concentrations of the crude acetone extracts and the PKC inhibitor, chelerythrine chloride, were added to the Petri dishes before dark treatment. In sterile water (control), large numbers of zoospores (ca. 1×10^6 per ml) were released and swam for 10–12 h in a helical fashion. Zoospore release was reduced by some of the crude acetone extracts and chelerythrine chloride and released zoospores swam very slowly or lysed depending on the concentration of the test materials (Table 1). Among the tested crude extracts, *Nigella sativa* (Kalijira) most effectively suppressed zoosporogenesis, followed by *Psidium guajava* (Guava) and *Duranta plumeri* (Duranta) at 100 µg/ml. Mean zoospore release in the presence of *N. sativa* extracts was 40% (relative to the control at 100%) with a 5% lysis of the released spores at 100 µg/ml. The same concentration of *P. guajava* extract resulted in 60% zoospore release but 25% of zoospores became encysted and others moved slowly. The release of zoospores remained unaffected by extracts up to 100 µg/ml of *Solanum sisymbriifolium* (Kanta Begun), *Piper betle* (Betel leaf), *Papaver nigrum* (Poppy), *Lawsonia inermis* (Hena), and *Eucalyptus* spp. (Eucalyptus). Zoospore release was almost unaffected by all the tested plant crude extracts at 20 and 50 µg/ml. The PKC inhibitor, chelerythrine chloride, effectively suppressed zoosporogenesis at 0.1 µg/ml with only 30% zoospore release at this concentration. Motility of the released zoospores was impaired, with 20% becoming round cystospores (Table 1).

***Motility inhibitory and lytic activities of plant extracts against** P. capsici **zoospores**.* Motility of zoospores is considered critical for the disease cycle of *P. capsici* and other zoosporic peronosporomycete phytopathogens. Of the 100 plant species tested, 19 severely inhibited zoospore motility (≥ 70% of zoospores were slowed or stopped moving) and 37 plant species showed moderate to low inhibitory activity towards zoospore motility (≥ 39–69%) when subjected to 20-fold diluted water extracts (Table 2). Water extracts of *Terminalia bohera* were most potent, impairing motility of 90% of the zoospores and subsequently causing lysis (Figure 1) within 60 min of treatment. Water extracts of *Ocium gratissimum* (Ram Tulsi) reduced motility of 85% of the zoospores but in contrast, *O. sanclum* extracts impaired just 35%. Interestingly, movement stopped and some zoospores lysed within 15 min in the presence of *O. gratissimum* extracts, although after 45 min of exposure, some cystospores germinated (5%) and formed germ tubes.

In sterile water, control zoospores remained motile for up to 10–12 hours. Motility inhibition followed by lysis of zoospores by the water extracts of *T. bohera* (Bohera), *Aphanamixis polystachya* (Pithraj), *Iresine herbstii* (Blood Leaf), *Manihot esculenta* (Cassava), *Vitis* sp. (Grape), *Phyllanthus emblica* (Amloki), *Diospyros blancoi* (Velvet apple), *Cajanus cajan* (Pigeon pea), *Chrysanthemum* sp. (Chrysanthemum), and *P. betle* (Betel leaf) is a key finding of the current investigation (Figure 2). Among the extracts, the highest lysis of zoospores was caused by *T. bohera* (80%) and by *Vitis* sp. (70%) at 60 min, while *M. esculenta* (60%), *D. blancoi* (50%), *Chrysanthemum* sp. (45%), and *P. emblica* (40%) showed moderate lysis at 60 minutes. The lowest lysis was observed by *I. herbstii* (30%), *C. cajan* (30%), *P. betle* (35%), and *A. polystachya* (25%) after 60 minutes.

The PKC inhibitor, chelerythrine chloride, inhibited motility followed by lysis of the immobile zoospores in a dose-dependent manner. It caused the highest lysis of zoospores (80%) at 0.2 µg/ml. For further quantitative assays, acetone extracts of 10 plants and chelerythrine chloride were used at a range of concentrations. Of these, only 4 acetone extracts inhibited zoospore motility (Table 3). Among them, *P. guajava* and *N. sativa* caused a 100% inhibition of motility (after 60 min) at 100 µg/ml followed by *M. esculenta* (70%) and *D. plumeri* (70%). Zoospore motility was also inhibited by the lower concentration of 20 µg/ml acetone extracts of *P. guajava*, *M. esculenta*, *N. sativa,* and *D. plumeri*, with the greatest

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Table 1. Effects of acetone extract of medicinal plants on the release of *Phytophthora capsici* zoospores from sporangia

Name of plant extracts/ compounds	Dose (µg/ml)	Relative percent of released zoospores (% ± SE)[a] and their behaviours	
		zoospores	behaviours of released zoospores
*Psidium guajava**	100	60 ± 3	slow moving and about 25% encysted zoospores
	50	70 ± 5	slow moving
	20	90 ± 7	normal swimming
Manihot esculenta	100	65 ± 2	slow moving and about 2% lysed zoospores
	50	70 ± 2	slow moving
	20	90 ± 3	normal swimming
Lawsennia iermis	100	80 ± 5	normal swimming
	50	85 ± 8	normal swimming
	20	95 ± 3	normal swimming
*Nigella sativa**	100	40 ± 3	slow moving and about 5% lysed zoospores
	50	55 ± 9	slow moving
	20	85 ± 5	normal swimming
Papaver nigrum	100	85 ± 1	normal swimming
	50	85 ± 5	normal swimming
	20	95 ± 2	normal swimming
Eucalyptus sp.	100	80 ± 5	normal swimming
	50	80 ± 3	normal swimming
	20	98 ± 2	normal swimming
Duranta plumeri	100	65 ± 1	slow moving
	50	75 ± 3	slow moving
	20	90 ± 3	normal swimming
Piper betle	100	90 ± 8	normal swimming
	50	90 ± 3	normal swimming
	20	98 ± 2	normal swimming
Solanum sisymbrifolium	100	80 ± 7	almost normal swimming
	50	98 ± 5	normal swimming
	20	98 ± 3	normal swimming
Mimosa pudica	100	80 ± 3	slow moving
	50	85 ± 3	normal swimming
	20	95 ± 4	normal swimming
Chelerythrine* chloride	0.2	23 ± 2	slow moving, 20% encysted and about 2% lysed zoospores
	0.1	30 ± 0	slow moving and about 20% encysted zoospores
	0.01	65 ± 3	normal swimming
	0.01	78 ± 1.5	normal swimming
Control Zoospores		100 ± 0	quick swimming in a helical fashion

[a]data presented here are average values ± SE of at least three replications for each dose of compound; *highest performance

effect caused by *N. sativa* (50%) after 60 min, followed by *D. plumeri* (40%). Chelerythrine chloride was the most powerful motility inhibitor of *P. capsici* zoospores, inhibiting all zoospores both at 0.1 and 0.2 µg/ml after 60 minutes. In the case of the control treatment (1% DMSO/acetone), all zoospores moved normally.

Generalised Linear Regression using plant extract concentration and time as explanatory variables of the data in Table 3 confirmed that increasing extract concentration resulted in significantly greater degrees of inhibition (concentration as a single explanatory variable) for each species apart from *N. sativa* (Table 4). A concentration × time interaction suggests

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Table 2. Motility inhibitory effects of the 20-fold diluted water extract of medicinal plants against *Phytophthora capsici* zoospores

Scientific name (Common name)	Plant parts	Motility inhibition (%) of *P. capsici* zoospores	Scientific name (Common name)	Plant parts	Motility inhibition (%) of *P. capsici* zoospores
Terminalia bohera (Bohera)	fruit	90	*Vitex negundo* (Ohaste tree)		10
Nigella sativa (Kalijira)	seed	95	*Elettaria cardamomum* (Cardamom)		10
Papaver nigrum (Poppy)	flower	90	*Jasminum pubescens* (Jesmine)		15
Vitis sp. (Grape)		85	*Hopea odorata* (Rock Dammar)		15
Piper betle (Betel leaf)		78	*Gmelina arborea* (Coomb teak)		25
Duranta plumeri (Duranta)		80	*Datura innoxia* (Thorn apple)		35
Manihot esculenta (Cassava)		80	*Syzygium jambos* (Rose apple)		10
Iresine herbtii (Blood leaf)	leaf	85	*Calendula officinalis* (Marigold)		10
Eucalyptus spp. (Eucalyptus)		80	*Tabernaemontana coronaria* (Tagar)		30
Phyllanthus emblica (Amloki)		92	*Mimusops elengi* (Bakul)		25
Cajanus cajan (Pigeon pea)		80	*Terminalia chebula* (Haritaki)		25
Psidium guajava (Guava)		90	*Murraya koenigii* (Curry leaf)	leaf	20
Lawsonia inermis (Hena)		80	*Cynodon dactylon* (Durba)		10
Solanum mauritianum (Wild tobacco)		75	*Myrciaria cauliflora* (Jabortikaba)		15
Solanum sisymbrifolium (Kanta begun)	aerial	70	*Couroupita guianensis* (Naglingo)		20
Mimosa pudica (Lojjaboti)		80	*Withania somnifera* (Ashwagondha)		15
Ocimum gratissimum (Ram tulsi)		85	*Ipomoea batatas* (Sweet potato)		20
Moringa oleifera (Drumstick)	bark	75	*Terminalia arjuna* (Arjun)		30
Cedrus deodara (Debdaru)		70	*Cassia alata* (Ringworm shrub)		10
Diospyros blancoi (Velvet apple)		60	*Epipremnum aureum* (Money plant)		30
Piper longum (Long pepper)		50	*Holarrhena antidysenterica* (Khurchi)		20
Magnolia grandiflora (Magnolia)		45	*Madhuca indica* (Mohua)		30
Aphanamixis polystachya (Pithraj)	leaf	40	*Stereospermum suaveolens* (Trumpet)		15
Chrysanthemum sp. (Chrysanthemum)		60	*Calotropis gigantea* (Calotropis)		15
Jasminum sambac (Arabian jasmine)		50	*Coracias benghalensis* (Indian roller)		15
Ipomoea carnea (Dhol kolmi)		65	*Asparagus racemosus* (Indian asparagus)	aerial	15
Allamanda cathartica (Yellow bell)	aerial	45	*Ocimum sanctum* (Tulsi)		35
Pterocarpus santalinus (Red sandawood)	leaf	25	*Clitoria ternatea* (Aparajita)		10

Data are shown here over control

that zoospore motility, when subjected to *N. sativa* extracts, was least affected between 30 and 45 min at the 50 and 20 µg/ml dose. The lack of a statistically significant concentration effect (at each individual time or across all times) shows that even low doses of the *N. sativa* extracts effectively reduce zoospore motility.

DISCUSSION

Zoosporogenesis and motility are two critical events in the disease cycle of *P capsici*. In this study, we demonstrated that crude extracts of some medicinal plant extracts from Bangladesh and a selective inhibitor of the PKC, chelerythrine chloride, markedly suppressed zoosporogenesis, and impaired motility of *P. capsici* zoospores. Although suppression of zoosporogenesis and inhibition of zoospore motility of some peronosporomycete phytopathogens such as *A. cochlioides* and *P. viticola* by medicinal plant (non-host) extracts has been reported (Islam *et al.* 2004; Islam 2013), the current work demonstrates for the first time the inhibitory effects of a large number of medicinal plant extracts and a selective

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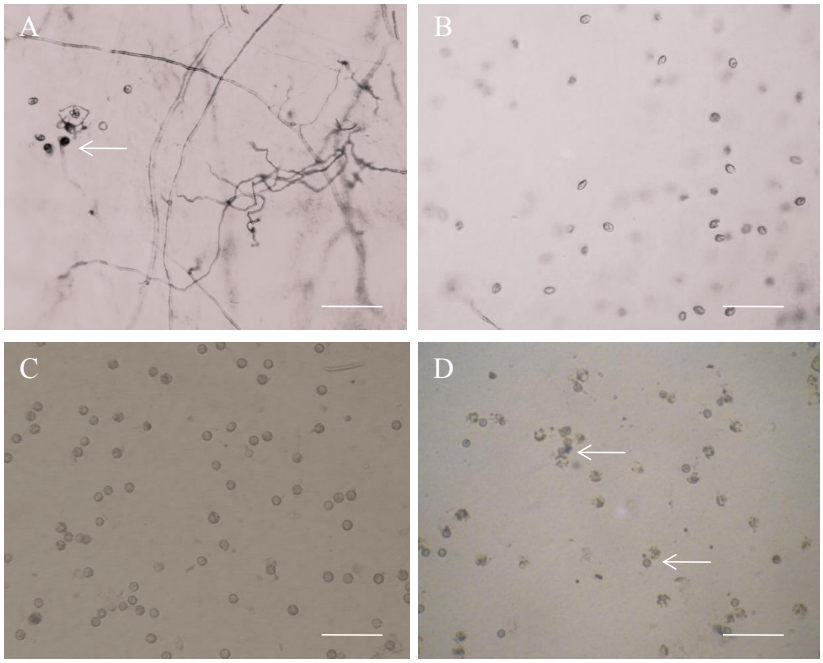

Figure 1. Effect of water extracts of *Terminalia bohera* seed (20-fold diluted) on motility of *P. capsici* zoospores. (**A**) zoosporogenesis (white arrow); (**B**) normal moving zoospores (control); (**C**) halted zoospores (cystospores), and (**D**) lysed zoospores (white arrow). White bars indicate 50 μm

inhibitor of the PKC against *P. capsici*. It is likely that secondary metabolites present in the crude extracts of the medicinal plants are involved in these interesting biological activities since inhibition of zoospore motility by secondary metabolites from medicinal plants and microorganisms has previously been reported (Islam *et al.* 2002, 2004; Islam & Hossain 2013).

In the current study, 20-fold diluted water extracts of 19 plants (*M. pudica*, *S. sisymbriifolium*, *S. mauritianum*, *Eucalyptus* sp., *C. deodara*, *P. nigrum*, *N. sativa*, *L. inermis*, *P. guajava*, *M. oleifera*, *M. esculenta*,

Table 3. Motility inhibitory activity of plant crude acetone extracts against *Phytophthora capsici* zoospores

Name of extracts/ compound	Dose (μg/ml)	Motility inhibitory activity (% ± SE)[a]			
		15 min	30 min	45 min	60 min
*Psidium guajava**	100	60 ± 0	65 ± 2	80 ± 7	100 ± 0
	50	10 ± 0	20 ± 2	35 ± 4	45 ± 3
	20	5 ± 0	10 ± 0	20 ± 0	20 ± 0
Manihot esculenta	100	40 ± 0	45 ± 3	50 ± 1	70 ± 3
	50	10 ± 1	15 ± 0	20 ± 0	30 ± 1
	20	2 ± 0	5 ± 0	10 ± 0	10 ± 0
*Nigella sativa**	100	30 ± 0	50 ± 1	60 ± 2	100 ± 0
	50	20 ± 0	40 ± 0	45 ± 0	60 ± 2
	20	10 ± 0	30 ± 0	40 ± 0	50 ± 2
Duranta plumeri	100	50 ± 1	50 ± 2	65 ± 2	70 ± 2
	50	25 ± 0	30 ± 1	45 ± 2	50 ± 1
	20	20 ± 0	25 ± 1	30 ± 1	40 ± 2
*Chelerythrine chloride**	0.2	72 ± 2	87 ± 2	100 ± 0	100 ± 0
	0.1	47 ± 3	77 ± 2	86 ± 2	100 ± 0
	0.01	0 ± 0	18 ± 2	30 ± 0	37 ± 2
	0.001	0 ± 0	0 ± 0	2 ± 0	5 ± 0
Control zoospores	–	0 ± 0	0 ± 0	0 ± 0	0 ± 0

*100% zoospores were completely immobile or moved very slowly after 60 min

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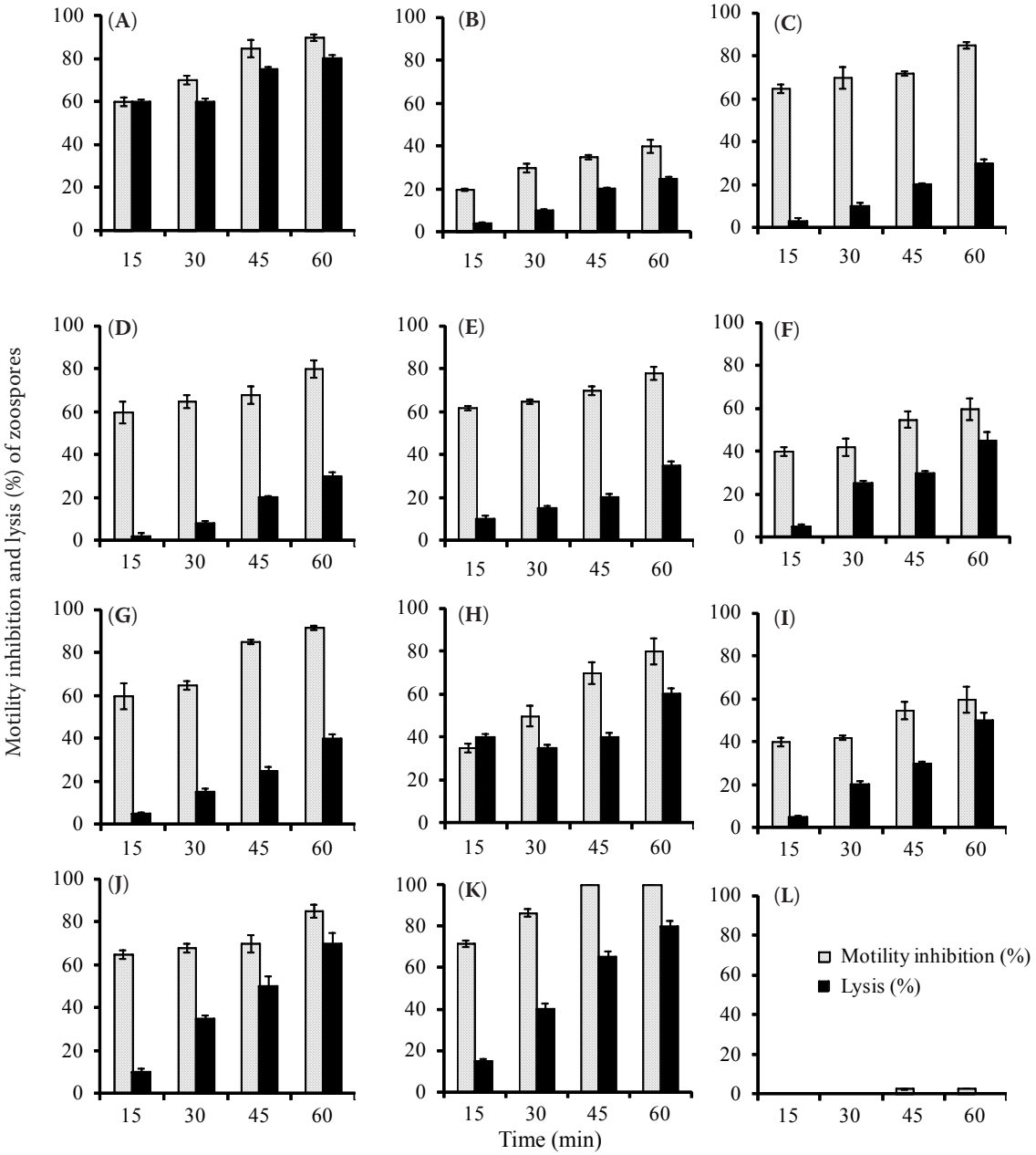

Figure 2. Quantitative evaluation of inhibition of motility and lytic activities of 20-fold diluted plant extracts in water (**A**–**J**) and 0.2 µg/ml of a PKC inhibitor chelerythrine chloride (**K**) against *P. capsici* zoospores: (**A**) *Terminalia bohera*; (**B**) *Aphanamixis polystachya*; (**C**) *Iresine herbstii*; (D) *Cajanus cajan*; (**E**) *Piper betle*; (**F**) *Chrysanthemum* sp.; (**G**) *Phyllanthus emblica*; (**H**) *Manihot esculenta*; (**I**) *Diospyros blancoi*; (**J**) *Vitis* sp.; (**K**) Chelerythrine chloride; (**L**) control (water only) zoospores

D. plumeri, *P. betle*, *Vitis* sp., *P. emblica*, *T. bohera*, *C. cajan, O. gratissimum* L., and *I. herbstii*) impaired motility (> 70%) of *P. capsici* zoospores. In addition, acetone extracts of two plants (*P. guajava* and *N. sativa*) and the PKC inhibitor, chelerythrine chloride, completely inhibited motility of all *P. capsici* zoospores (Table 3). Chelerythrine chloride and crude acetone extracts of all the plants listed in Table 3 inhibited motility with *N. sativa* appearing to be the most effective of the extracts across each concentration tested. Interestingly, chelerythrine chloride at higher concentrations showed lytic activity against *P. capsici* zoospores. It arrested motility of *P. capsici* zoospores at a concentration starting from 0.01 µg/ml. To develop a management strategy against *P. capsici*, 100% halting of zoospores without regeneration is

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Table 4. Generalised Linear Regression for the inhibition of motility by plant crude acetone extracts (percentage data are shown in Table 3)

Factors/Probability	*Psidium guajava*	*Manihot esculenta*	*Nigella sativa*	*Duranta plumeri*	Chelerythrine chloride
Concentration	$P = 0.001$	$P = 0.002$	ns	$P = 0.021$	$P = 0.006$
Time	ns	ns	ns	ns	ns
Concentration × time	$P = 0.005$	$P = 0.003$	$P = 0.017$	ns	ns

ns – not significant

desirable. In an earlier report, we demonstrated that an indolocarbazole antibiotic, staurosporine, and chelerythrine chloride suppressed zoosporogenesis and inhibited motility of *A. cochlioides* and *P. viticola* zoospores (Islam *et al*. 2011). Furthermore, the application of micromolar concentrations of a PKC inhibitor also completely inhibited downy mildew disease in grapevine (Islam *et al*. 2011). The inhibition of PKC and growth of *Staphylococcus aureus* and many other human pathogens by chelerythrine have been reported (Tavares *et al*. 2014). This is the first report on the suppression of zoosporogenesis and motility impairment of *P. capsici* zoospores by the PKC inhibitor, chelerythrine chloride. Several previous studies demonstrated that formulated plant extracts or plant oils suppress diseases caused by *Phytophthora* species (Bowers & Locke 2004; Islam & Hossain 2013). In the present study, both water and acetone extracts of *N. sativa* seeds showed the greatest inhibitory effects against zoospore motility; interestingly, the seeds of this plant are used in herbal remedies and show pharmacological properties.

An important finding of this study was that 20-fold diluted water extracts of *D. plumeri* and *M. esculenta* instantly stopped zoospore motility and subsequently caused lysis of zoospores. In addition, *M. pudica*, *S. sisymbrifolium*, *S. mauritianum*, *Eucalyptus* sp., *C. deodara*, *P. nigrum*, *L. inermis*, *P. guajava*, *M. oleifera*, *P. betle*, *Vitis* sp., *D. blancoi*, *P. emblica*, *T. bohera*, *C. cajan, O. gratissimum* L., and *I. herbstii* also impaired motility of zoospores. Motility inhibition of *P. capsici* zoospores by these non-host medicinal plant extracts has not been previously reported. However, it is known that extracts of garlic and clove and to a lesser extent leaf extracts of *Duranta*, *Azadirachta indica,* and *Lantana* inhibit *P. capsici (*Masuduzzaman *et al. 2008).* Similarly, Allamanda leaf extract and its chromatographically separated fractions inhibited mycelial growth of *P. capsici* (Mone *et al*. 2013). The zoospore motility inhibitor(s) from these plant extracts have not been identified. Islam *et al*. (2011) confirmed that the suppression of zoosporogenesis and inhibition of motility of *P. viticola* zoospores by indolocarbazole alkaloids isolated from *Streptomyces* spp. is linked to the inhibition of PKC in the cells of *P. viticola*. Our current findings confirm for the first time that the PKC inhibitor chelerythrine chloride also suppressed zoosporogenesis and motility of *P. capsici* zoospores. A further bioassay-guided chromatographic study of zoospore motility inhibiting plant extracts is needed to discover novel metabolites useful for managing plant diseases caused by *P. capsici*.

Another interesting observation in our study was that, water extracts of *T. bohera*, *A. polystachya*, *I. herbstii*, *M. esculenta*, *Vitis* sp., *P. emblica, D. blancoi*, *C. cajan*, *Chrysanthemum* sp., and *P. betle* caused lysis of zoospores. It was reported earlier that a secondary metabolite (avenacin A1) contained in root extracts of *Avena sativa* caused lysis of *Pythium* zoospores (Deacon & Mitchel 1985). Extracts of *Thuja plicata, Calocedrus decurrens, Chameacyperis nootkatensis* have also been reported to cause lysis of *P. ramorum* zoospores (Manter *et al*. 2006). Similarly, polyflavonoid tannins in *Lannea coromandelica* bark also cause lysis of *A. cochlioides* zoospores (Islam *et al*. 2002). Lysis of *P. capsici* zoospores by several medicinal plant extracts of Bangladeshi origin shown in this paper has not previously been reported. The toxic effect of these plant extracts on *P. capsici* leads to lysis of zoospores and raises the possibility of suppression of *P. capsici* in the field. Strong lytic activities shown by water extracts of *T. bohera* seed in this report merits further investigation to elucidate the active component present within the extracts.

In conclusion, this study identified some Bangladeshi medicinal plant extracts that effectively suppress zoosporogenesis and impair motility of *P. capsici* zoospores. Among the tested plant extracts *Terminalia bohera*, *Nigella sativa*, *Ocimum gratissimum*, *Duranta plumeri*, and a PKC inhibitor chelerythrine chloride displayed potent inhibitory effects against

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motility and caused subsequent lysis of *P. capsici* zoospores. Although the selective inhibitor of PKC suppressed zoosporogenesis and motility of *P. capsici* zoospores, we cannot exclude the possibility of any other mechanisms involved in the inhibitory activities of the plant extracts highlighted in this study. As the suppression of zoosporogenesis and inhibition of zoospores motility eliminate the chance of infection by zoosporic phytopathogens, some inhibitory plant extracts identified in this research might be useful for controlling the disease caused by *P. capsici*. The isolation of active components in these plant extracts could lead to interesting secondary metabolites that might be useful biopesticides against *P. capsici*, in addition to elucidating the modes of action of the extracts and inhibitory activities outlined.

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