

1. INTRODUCTION

Pointed gourd or *Trichosanthes dioica* Roxb., is a genus of family cucurbitaceae. The plant is annual or perennial herb distributed in tropical Asia and Australia. Pointed gourd is thought to be originated in the Indian subcontinent or Indo-Malayan region (Singh and Whitehead, 1999; Rai *et al.*, 2008; Mythili and Thomas 1999; Nayak *et al.*, 2016). *T. dioica* is one of the most consumed species of *Trichosanthes* genus in the Asian tropical countries particularly in Bangladesh and India (Kumar and Singh, 2011). Major pointed gourd cultivating countries are Bangladesh, India, Pakistan, Myanmar, Nepal and Sri Lanka (Renner and Pandey, 2013; Mehta and Sharma, 2012). In India it is cultivated throughout the plain of Northern India, including Assam and Bengal. Kirtikar and Basu (2001) reported some common names of pointed gourd as parwal, palwal, parmali, patol, and potala. About 316,000 metric tons of pointed gourd was cultivated on 20,000 hectares of land in India during 2017–18 (Ministry of Agriculture, Government of India, 2019). The fruits and leaves (the main edible parts of the plant) are cooked in various ways (Singh and Whitehead, 1999). The plant is dioecious, and grows as vines that are thick like a pencil. The leaves are dark green, rough on both surfaces, ovate, cordate, oblong and rigid. Roots are tuberous with long tap root system (Maurya, 1985).

The various chemical constituents present in pointed gourd are Vitamin A, Vitamin C, Saponins, Tannins, alkaloids, mixture of novel peptides, tetra and pentacyclic triterpenes (Chopra *et al.*, 2002; Ghaisas *et al.*, 2008). The seed extract of this fruit contain 7-oxidihydrokaro undiol-3-benzoate as the most predominant component in the highly polar fraction of the non saponifiable lipid (Toshihiro *et al.*, 1997). Two main phytosterols namely 24 α -ethyl cholest-7-enol and 24 β -ethyl cholest-7-enol are present in this plant (Kongton, 2003). Roots contain a phytosterol, hentriacontans, an amorphous saponin, and a non-nitrogenous bitter principle (Gupta and Pagoch, 2014). Several bioactive compounds are also present including peptides (*viz.* trichosanthin and lectin); triterpenes (*viz.* cucurbitacin B,

euphol, α -amyrin, β -amyrin, betulin, taraxerol, lupeol and karounidiol), sterols, steroidal saponin and flavonoids (Khandakera *et al.*, 2018).

Pointed gourd has several therapeutic properties. Those are antihyperglycemic activity (Rai *et al.*, 2008), hepatoprotective activity (Ghaisas *et al.*, 2008), cholesterol-lowering activity (Sharmila *et al.*, 2007), antiinflammatory activity (Fulzule *et al.*, 2001), in skin disorder protective (Bhujbal, 1999), antioxidant activity (Shivhare *et al.*, 2010; Dixit and Kar, 2009), wound healing activity (Shivhare *et al.*, 2010a; Shivhare *et al.*, 2010b), antitumor activity (Bhattacharya *et al.*, 2011), ameliorative effect on arsenic toxicity (Bhattacharya *et al.*, 2012; Bhattacharya *et al.*, 2013-2014), immunomodulatory effect (Bhadoriyal and Mandoriya, 2012), antipyretic activity (Alam *et al.*, 2011), and neuropharmacological activity (Bhattacharya, 2012; 2013).

Several diseases have been reported to cause considerable damage to pointed gourd production in India. These include downy mildew caused by *Pseudoperonospora cubensis* (Bilgrami *et al.*, 1979; Khatua *et al.*, 1981; Mondal *et al.*, 2014), fruit rot by *Pythium aphanidermatum* and *P. cucurbitacearum* (Chaudhuri, 1975), sclerotinia stem rot by *Sclerotinia sclerotiorum* (Khatua *et al.*, 2014), fruit and vine rot by *Phytophthora melonis* (Khatua *et al.*, 1981; Guharoy *et al.*, 2006), anthracnose by *Colletotrichum capsici* (Khatua, 2004) and root knot nematode by *Meloidogyne incognita* (Mukherjee & Sharma, 1973; Khatua, 2004). Raj *et al.* (2011) have also reported *Ageratum enation virus* infection of pointed gourd in India.

Plant pathogenesis related (PR) proteins are structurally diverse group of plant protein that plays an important role in disease resistance and are generally induced by various types of pathogens such as viruses, bacteria, and fungi (van Loon 1985, Rigden *et al.*, 1988, Lamb *et al.*, 1989) and (Mahendranathan *et al.*, 2016). When a pathogen attacks a plant they generate stress, for that, PR proteins are produced in a high concentration. They are present in plant cells intercellularly and intracellularly (Agrios, 2005). Several groups of PR proteins have been classified according to their properties and functions. Currently PR proteins are categorized into 17

families according to their properties and functions, including β -1,3-glucanases, chitinases, thaumatin-like proteins, peroxidases, ribosome-inactivating proteins, defensins, thionins, nonspecific lipid transfer proteins, oxalate oxidase, and oxalate-oxidase-like proteins (van Loon and van Strien, 1999). PR proteins are either extremely acidic or extremely basic and therefore are highly soluble and reactive (Legrand *et al.*, 1987). The signal compounds responsible for induction of PR proteins include salicylic acid, ethylene, xylanase, polypeptide systemin, jasmonic acid and probably some others (Agrios, 2005).

Apart from PR proteins (peroxidase, chitinase, β -1,3- glucanase etc.) some plant enzymes such as polyphenol oxidase, phenylalanine ammonia lyase are also related to resistance inducement in plants (Prasannath and De Costa, 2015; Gajanayaka *et al.*, 2014; Seneviratne *et al.*, 2014). Phenylalanine ammonia-lyase is the first enzyme of the phenylpropanoid pathway and is involved in the biosynthesis of lignins, phenolics and phytoalexins which increase the plant defense (Pellegrini *et al.*, 1994; Walters *et al.*, 2005).

Priming is a physiological state of plant in which it responds more rapidly to the exposure of biotic or abiotic stress (Conrath, 2009). Infection by necrotising pathogen, colonisation of microbes in the roots, or after treatment with various chemicals, many plants establish a unique physiological situation that is called the 'primed' state of the plant. In the primed condition, plants are able to 'recall' the previous infection, root colonisation or chemical treatment. In primed state plants respond more rapidly and/or effectively when re-exposed to biotic or abiotic stress, a feature that is frequently associated with enhanced disease resistance (Goellner and Conrath, 2008). Priming initially triggers a minor part of a defense response that increases the plant's ability to defend itself against future antagonists. Priming agents may be living organisms, chemicals or components thereof, and priming can be applied to various tissues and at diverse developmental stages (Westman, 2019).

Though molecular mechanisms about priming are not completely understood, but there is a consensus that primed plants conserve a memory and two potential mechanisms have been suggested. One involves accumulation of mitogen-activated protein kinases (MPKs) (Beckers *et al.*, 2009). The other mechanism is that epigenetic changes in DNA methylation and histone modifications which may be the carriers of stress memories and triggers the immune responses (Conrath, 2011; Jaskiewicz, 2011; Espinas, 2016).

Till date several chemicals have been established as successful inducers such as 2,6-dichloroisonicotinic acid (INA), salicylic acid (SA) and its synthetic analogs, chitin, lipopolysaccharide (LPS), benzo (1,2,3) thiadiazole-7-carbothioic acid (BTH), azelaic acid, β -aminobutyric acid (BABA), Vitamin B1 (thiamine), Vitamin B2 (riboflavin) hexanoic acid, and many other natural or synthetic compounds (Conrath, 2002; Ahn *et al.*, 2005; Conrath *et al.*, 2006; Zhang *et al.*, 2008; Jung *et al.*, 2009; Scalschi 2013). Application of these chemicals results as 'priming activation' by various cellular defense responses in plants. These cellular responses are included in rapid induction of ion transport changes at the plasma-membrane, early reactive oxygen species (ROS) burst, synthesis and secretion of phytoalexins, callose deposition and the accumulation of transcripts for various pathogenesis-related (PR) genes (Conrath, 2002; Goellner and Conrath, 2008). Most of the above mentioned activated defense responses were identified to generally dependent on some known resistance signaling pathways, such as systemic acquired resistance (SAR) and induced systemic resistance (ISR). These pathways use endogenous hormone salicylic acid (SA) and jasmonic acid (JA) as the signal transduction molecules, respectively. However, the activated plant defense by β -aminobutyric acid (BABA) was reported to mediate another signaling mechanism that differs from SAR and ISR (Ton *et al.*, 2004).

Diseases caused by fungi are affecting the production and quality of a wide range of cucurbits at an alarming rate. Many reports of fungal diseases are available in cucurbitaceae occurring throughout the world. But

information of fungal diseases of pointed gourd is scanty. Establishment of successful disease control requires a good understanding of plant-pathogen interactions along with understanding of environmental conditions required for disease establishment. Understanding the molecular responses associated with host defence mechanism in pointed gourd is very important for better management of the crop. The resistance mechanisms and the major genes involved in the defense system of pointed gourd are quite unknown.

On the basis of above information it was considered worthwhile to isolate and identify the pathogens affecting pointed gourd and also to know about the resistance mechanisms along with the major genes involved in the defense system of pointed gourd.

Thus, the present study has been taken into consideration with the following objectives.

Objectives:

1. Isolation of major pathogens responsible for foliar and fruit diseases in *Trichosanthes dioica* available in sub-Himalayan West Bengal.
2. Pathogenicity tests of the isolated pathogens and assessment of disease incidence.
3. Physiological, morphological and molecular characterization of some pathogens.
4. Studies on disease resistance of *Trichosanthes dioica*.
5. Induction of plant defense with some signalling molecules against major isolated pathogen.

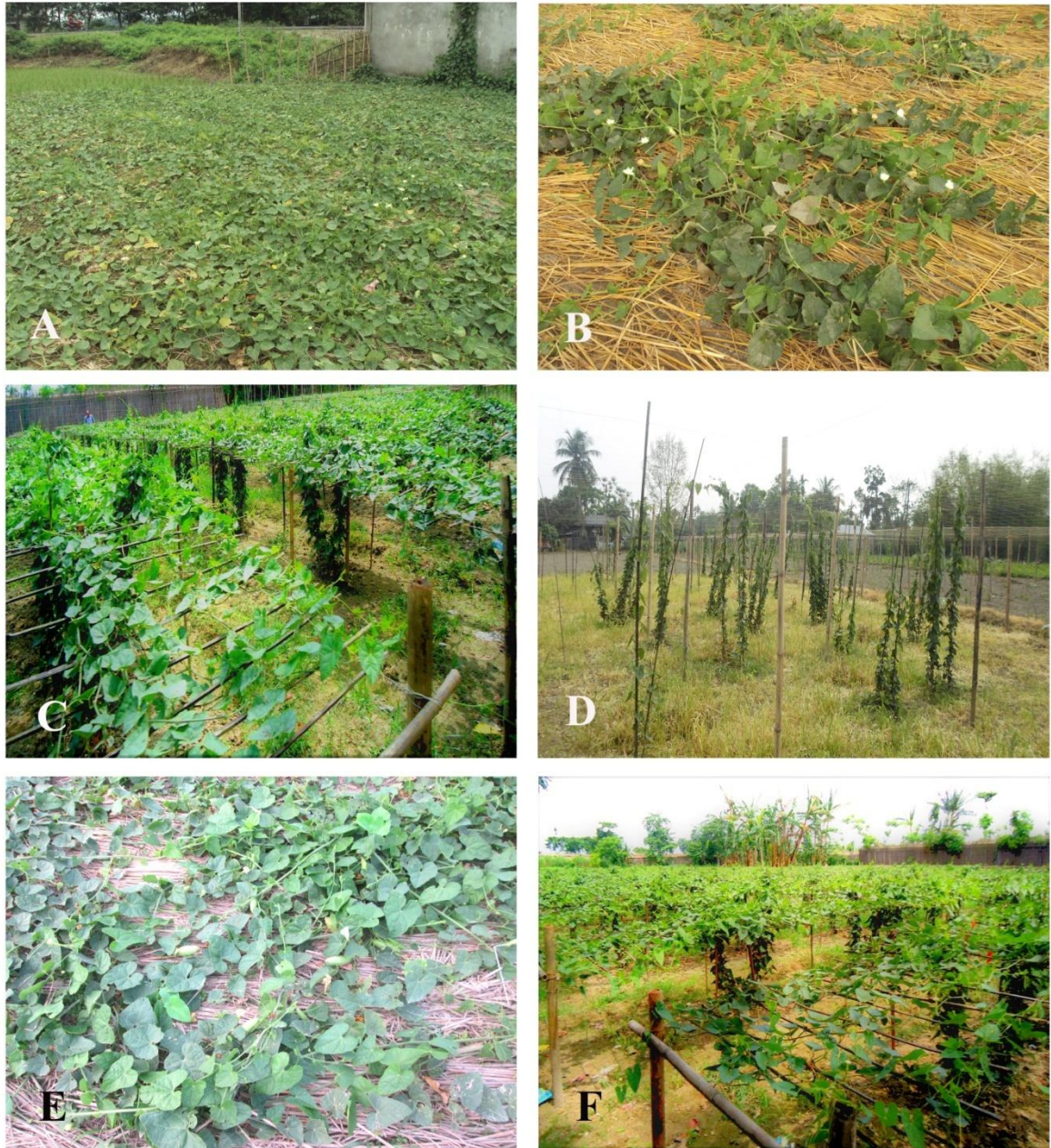


Fig. 1.1: Cultivated pointed gourd plants in farmer's field in different places of northern West Bengal: A. Kharibari, Siliguri subdivision of Darjeeling District. B. Islampur, Uttar Dinajpur District. C. Dhupguri, Jalpaiguri District. D. Guabari, Siliguri subdivision of Darjeeling District. E. Alipurduar, Alipurduar District. and F. Tambari, Siliguri subdivision of Darjeeling District.