CHAPTER ONE

INTRODUCTION

Green cumin (*Cuminum cyminum* L.), is believed to be a native from the East Mediterranean to East India. It is a herbaceous and medicinal crop and one of the oldest and popular seed spice worldwide after black pepper (Agarwal, 1996). In fact, it is one of the most important crops in terms of exports, income, water use efficiency and reclamation of the arid and semiarid regions (Tuncturk and Tuncturk, 2006). The estimated world production is around 300,000 tons (Divakara Sastry & Anandaraj, 2013).

Although cumin was originally cultivated in Iran, where it is one of the most important export crops, and Mediterranean region but today it is also grown in Uzbekistan, Tazikistan, Turkey, Morocco, Egypt, India, Syria, Mexico, Bulgaria, Cyprus and Chile where India is the largest producer and consumer of cumin seed in the world (Kafie *et al*., 2002).

Cumin seed is generally used as a spicy food in the form of powder for imparting flavor to different food preparations (Kafie *et al*., 2002). It also has a variety of medicinal properties (Avatar *et al*., 1991 and Weiss, 1996).

In the Sudan, Cumin spread up the Nile valley where it continues to be sown by stallholders in the winter season to provide flavoring and other parts of the country. The production is between 0.9 – 1.4 tons in 2013 – 2014 (Ministry of agricultural statistic, 2014).

The major constraints facing the production of cumin worldwide are losses caused by diseases, insects, and weeds. In fact, diseases can become damaging as to preclude cumin cultivation in a particular region such as Gujarat in India (Jansen, 1981). Among these, the most important are fungi e.g. leaf blight caused by *Alternaria burnsii* (Chand *et al*., 1999), *Fusarium oxysporum f. sp. cumini* that cause wilt in cumin (Omer *et al*., 1997) and powdery mildew caused by *Erysiphe polygoni* (Weiss, 2002).
In Sudan, although cumin is a medicinal plant that is used in food industries, drinks, cosmetics, soap, perfume and cheese (Hajlaoui, *et al*., 2010), and the cultivation of the crop is widely spreading specially in the Northern parts of Sudan, but very few or no attention was given by research to the plant protection from diseases. In fact, powdery mildew caused by *Erysiphe polygoni* is one of damaging pathogen limiting the productivity of this crop (personal communication)

Moreover, and in most cases, in order to prevent the plant diseases and to protect the crop plants against pathogens, chemical control methods are in practice. However, although the use of chemicals has increased of yields obtained (Ali, 1996), but one of the major problems with the constant use of chemicals is that resistance can be induced in target organisms in addition to contamination of the environment with very toxic substances (Carvalho, 2004 and Okigbo, 2004). This has initiated the exploration of safe alternate products.

Historically, the presence of antimicrobial compounds in higher plants has been recognized as important products in combating plant pathogenic diseases. Such compounds, being biodegradable and selective in their toxicity and are considered valuable for controlling some plant diseases (Schmutterer, 2002). Recently, encouragement of use of safe alternate products to reduce pathogen inoculum in combination with proper cultural practices and the use of resistant varieties was emphasized by many research workers (Saxina *et al.,* 1981).

Obviously, no single approach for powdery mildew control proved to be effective and without drawback. Therefore, integrated management strategies are the only solution to maintain plant health. These strategies should include minimum use of chemicals for checking the pathogen population, and safe alternate antimicrobial compounds of higher plants.

Based on the foregoing, this study was undertaken to focus on investigation of two components for control of powdery mildew in cumin caused by *Erysiphe polygoni*, higher plant extracts and synthetic fungicides under field conditions in order to formulate promising disease management approach with following objectives:-

- To investigate the effect of different levels of Argel extract on incidence and severity of powdery mildew.
- To study the effect of different levels of NaHco3 salt on incidence and severity of powdery mildew.

- To evaluate the efficacy of systemic fungicide on control of the fungus.
- To evaluate the role of these different methods of control in combating this pathogen and on yield of cumin.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cumin

2.1.1. Classification:-

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Apiales

Family: Apiaceae (Umbelliferae)

Genus: *Cuminum*

Species: *C. cyminum* (Linn)

Cumin is an annual umbelliferous plant commonly cultivated in arid and semiarid regions and which believed to be a native from the east Mediterranean to east India (Agarwal, 1996). The crop is generally grown in sandy loam to clay soils during the winter season using irrigation (Lodha, 1995). Despite the relative importance of this medicinal plant in crop rotation of arid and semiarid regions and many advantages such as low water requirements exports, it has not been adequately studied and there is no much information on potential yield of the current cultivated plant worldwide (Katan *et al.,* 2011).

2.1.2 Historical Background

Cumin seeds excavated at the Syrian site Tell ed-Der, have been dated to the second millennium BC. Originally, Cumin was cultivated in Iran and Mediterranean region, today it is mostly grown in Iran, Uzbekistan, Tazikistan, Turkey, Morocco, Egypt, India, Syria, Mexico, Bulgaria, Cyprus and Chile. It is the second most popular spice in the world after black pepper (Agarwal, 1996). Cumin is known under various names in different countries (Divakara Sastry & Anandaraj, 2013).

2.1.3 Plant Description

The cumin plant grows to 30–50 cm (12–20 inch) tall and is harvested by hand. It is an annual herbaceous, with a slender, glabrous, branched stem which is 20–30 cm (8–12 inch) tall and has a diameter of 3–5 cm (1 ¼–2 inch). Each branch has two to three sub-branches. All the branches attain the same height; therefore the plant has a uniform canopy. The stem is colored grey or dark green. The leaves are 5–10 cm (2–4 inch) long, pinnate or bipinnate, with thread-like leaflets. The flowers are small, white or pink, and borne in umbels. Each umbel has five to seven umbellts. The fruit is a lateral fusiform or ovoidachene 4–5 mm (⅙–⅕ inch) long, containing two mericarps each with a single seed. Cumin seeds have eight ridges with oil canals. They resemble caraway seeds, being oblong in shape, longitudinally ridged, and yellow-brown in colour, like other members of the umbelliferae family such as caraway, parsley and dill (Divakara Sastry & Anandaraj, 2013).

2.1.4 Uses

Cumin seeds are used as spice for their distinct aroma. The seed is a major ingredient of curry and chili powders, special bakery products, processed meats sausages, prepared condiments, pickles, soups and sauerkraut, and is an ingredients in certain teas (Weiss, 1996).

Cumin seeds contain 2.5-4.0% volatile oil and aldehydes or cuminol, which attributes to the aroma (Agarwal, 1996). It is also used as a spicy food in the form of powder for imparting flavor to different food preparations (Kafie *et al*., 2002). This is in addition to a variety of medicinal properties (Avatar *et al*., 1991 and Weiss, 1996).

In traditional medicine, seeds are mixed with other ingredients to treat diarrhea and colic, and ground seed or extracts are considered to be a stimulant, antispasmodic, carminative, diuretic and emmenagogue and incorrectly an aphrodisiac (Sallal and Alkofahi, 1996).

2.1.5 Processing

Cumin oil is usually obtained by steam distilling the milled spice, although hydro-diffusion gives a higher yield, and more recently by supercritical gaseous extraction, which is claimed to give oil closer to the aroma and taste of the spice (Eikani *et al.*, 1999). Cumin oil is sometimes adulterated with synthetic cumin aldehyde where the content of the oil has strong larvicidal, fungicidal and antibacterial activity. An unusually oil use is as a bird repellent to reduce the nuisance roofing birds on buildings (Clark, 1998).

In the Sudan, Cumin spread up the Nile valley where it continues to be sown by stallholders in the winter season to provide flavoring. In Ethiopia small-scale cultivation is widespread and fruits commonly available in local markets (Weiss, 2002). The production is between 0.9 – 1.4 tons in 2013 – 2014 (Ministry of agricultural statistic, 2014).

The major constraints facing the production of cumin worldwide are losses caused by diseases, insects, and weeds. In fact, diseases can become damaging as to preclude cumin cultivation in a particular region such as Gujarat in India (Jansen, 1981). Among these, the most important are fungi e.g. leaf blight caused by *Alternaria burnsii* (Chand *et al*., 1999), *Fusarium oxysporum f. sp. cumini* that cause wilt in cumin(Omer *et al*., 1997) and powdery mildew caused by *Erysiphe polygoni* (Weiss, 2002).

2.2 Powdery Mildew (*Erysiphe polygoni*)

Powdery mildew of umbelliferous crops; is widespread and occurs on numerous genera in Apiaceae. It is distinguished from other *Erysiphe* spp. by the numerous, short, irregularly branched appendages and elongate, cylindric conidia (Kapoor, 1967).

2.2.1 Classification

Kingdom: Fungi

Phylum: Ascomycota

Class: Archiascomycetes

Order: Erysiphales

Family: Erysiphaceae

Genus: *Erysiphe*

Species: *E.polygoni* (Agrios, 2005).

2.2.2 Symptoms

On cumin, in India, where the perfect state has not apparently been reported, the grey white lesion spread over the whole leaf surface, new leaves are infected as soon as they appear and other above ground parts of the plant are attacked (Uppal *et al*., 1932). Powdery mildews seldom don't kill their hosts but utilize their nutrients, reduce photosynthesis, increase respiration and transpiration, impair growth, and reduce yields 20 to 40% (Agrios, 2005).

2.2.3 Diagnosis

The most conspicuous feature of powdery mildew diseases usually is the appearance of mycelia and conidia formed on infected host tissue. Fungal colonies expand to form whitish, circular patches on the host that can develop a powdery appearance when large numbers of conidia are produced. Colonies may eventually coalesce and cover large areas of affected plant organs. As mycelia age they often become gray or brown and may cover the entire surface of leaves and stems. Affected host tissue frequently is stunted, distorted, discolored and in the case of affected fruit scarred or rosette. Sometimes, particularly late in the season, ascocarps can be seen with a hand lens or the naked eye. Ascocarps usually are 0.1–0.3 mm in diameter, nearly spherical, and, as they age, turn from yellow to dark brown or black (Braun, 1995).

2.2.4 Epidemiology

Powdery mildew affects all kind of plants, except gymnosperms, and is one of the most common and widespread plant diseases (Agrios, 2005). Fungi that cause powdery mildew are obligate parasites (Agrios, 2005). The disease is more common in warm and dry climates (Agrios, 2005). *Podosphaera xanthii* is predominant in many countries and *Golovinomyces cichoracearum* is common in temperate zones (Bardin *et al.*, 1999). Dry climate favours dispersal of spores, while humid climate favour spore germination (Agrios, 2005). As long as the relative humidity is high, the spores can be released, germinate and cause an infection even if there is no water film on the plant surface (Agrios, 2005).

Generally, powdery mildew is not only favored by dry atmospheric and soil conditions. Moderate temperatures, reduced light intensity, fertile soil and succulent plant tissues also promote the disease development (Sitterly, 1978). Powdery mildews do also develop better in shade than in full light. In Sweden, *G. cichoracearum* is most common during early season and develops favourably when the climate is dry, while *P. xanthii* dominates during summer when the humidity is higher (Jordbruksverket, 2009).

The mycelia of powdery mildew are completely external and grow only on the surface of plant tissues. Only haustoria penetrate the leaves (Robinson and Decker-Walters, 1997; Agrios, 2005). Because of the external mycelia these fungi are sensitive for environmental factors such as wind and heavy rains (Agrios, 2005). Therefore, the disease development is favoured by hot and dry weather (Agrios, 2005).

On the plant surface, the fungal mycelium produces short conidiophores and each conidiophore produces chains of conidia (Agrios, 2005). The conidia are round, ovoid or rectangular. The powdery mildew fungi may produce cleistothecia, containing asci, when environmental conditions become unfavourable or nutrients are lacking. The cleistothecia are tiny, pinhead-sized and spherical. At first they are white, but later will turn into yellow-brown and finally into black (Agrios, 2005). The fungus causing powdery mildew can overwinter in crop residues and perennial weeds (Seebold, 2010).

Ascospore germination is favored by absence of water and relative humidity below 20% (Sitterly, 1978). For conidia to germinate water is needed. Because of their high water content and extremely efficient water conservation system they use internal water from the vacuoles instead of water from an external source. Because of reduced air circulation and light intensities, higher temperatures and continuous cropping, powdery mildews are more serious in greenhouses than in field. Severe powdery mildew infections in greenhouse can result in the earlier formed chlorotic spots which later become necrotic (Sitterly, 1978).

2.2.5 Host ranges

Host ranges vary considerably among different species of Erysiphales. Some species are restricted to one host family or genus, while other species appear able to infect a wide range of hosts in many families. Knowledge of the host range of a particular powdery mildew fungus is essential to controlling a plant disease because alternative hosts can serve as a source of inoculum. Host-fungus indexes can be helpful sources of information about the likely host range for a particular powdery mildew fungus (Jarvis *et al*., 2002).

2.2.6 Life cycle

Life cycle of a powdery mildew fungus can provide important clues to epidemiology and disease control. A complete life cycle includes both asexual and sexual reproduction. The asexual state produces conidia (asexual spores). The sexual state includes the ascocarps (variously termed perithecia, cleistothecia, and chasmothecia) that contain asci with ascospores. When both states occur, ascocarps usually are important in perennation (survival in the absence of a live host) and disease establishment, whereas the conidial state serves to spread and intensify the disease (Braun, 1987).

Powdery mildews produce conidia in enormous numbers during the growing season, typically within 3–7 days after infecting the host under disease-conducive conditions. The rapid rate of asexual reproduction can lead to exponential growth of powdery mildew populations resulting in epidemics. Sexual reproduction of powdery mildew often is initiated in many plant species after flowering or late in the growing season. Ascocarps are resistant to low temperatures and drought, which enables the ascospores they contain to survive harsh conditions. Genetic recombination resulting from sexual reproduction can produce new genotypes resistant to fungicides or that display greater virulence than parental genotypes. Ascocarps also can initiate new epidemics (typically in the spring following the growing season during which they were formed in temperate regions) when, following a rainstorm or irrigation event, they discharge ascospores. Ascospores that land on susceptible and unprotected host tissue can establish primary infections to initiate the epidemic (Braun, 1995).

Powdery mildews also can survive winter in the form of dormant mycelium within buds of infected plants. In regions with mild winters, such as parts of the Pacific Northwest west of the Cascade Range, sexual states do not seem to occur frequently, or to be as important in over-wintering, as in areas that are inland. For non-deciduous plants in regions with mild climates powdery mildews can persist as mycelium on infected host tissue through the winter. (Braun *et al.,* 2002).

2.3 Control of powdery mildew

Numerous species of crops are susceptible to powdery mildew, but cumin is the most severely affected group. Cumin powdery mildew is one of the most important limiting factors for cumin production worldwide (Sharma and Khan, 1991; Abd-El-Kareem *et al*., 2004 and PÉREZ-GARCÍA *et al.,* 2009). Applying synthetic fungicides to control plant diseases has resulted in environmental pollution, phytotoxicity to the host and a buildup in resistance in plant pathogens to fungicides (McGrath *et al.,* 1996; and Ishii *et al.,* 2001). To reduce the harmful effects of fungicide, application of alternative control methods are required. Therefore, it was important to develop economic biological control procedures or materials with low toxicity for plants and of practicable use in agricultural applications. Foliar application has been used as a means of supplying supplemental doses of minor and major nutrients, plant hormones, stimulants, to improve drought tolerance, enhance crop quality, and control foliar diseases of plants (Yohalem *et al.,* 1994).

2.3.1 Cultural practices

Cultural practices should be utilized to minimize conditions favorable for infection and spread of powdery mildew and to improve spray deposits to foliage. These practices include:

• Orientating rows in the direction of prevailing winds to encourage flow-through of air, and using appropriate planting densities and trellises to help minimize crowded canopies.

• Using canopy management techniques to encourage air movement, the penetration of sunlight and spray penetration into the canopy.

• Careful use of water and fertilizer inputs, especially nitrogen, to minimize growth of large dense canopies (Nicholas *et al*., 1994).

2.3.2 Biological control

Biological control can be described as the suppression of damaging activities inflicted of a harmful organism by one or more other organisms (Pal and McSpadden, 2006). These suppressive activities are often conducted by natural enemies, when it concerns insect or arachnid control. In plant pathology the term biological control commonly applies to the phenomenon of microbial antagonism (Kiss, 2003). In some crops, such as tomato, biological control is used as an alternative to chemical control to prevent or suppress powdery mildews. Greenhouse environments are favourable for biological control, since conditions in the greenhouse can be optimized for the biocontrol agent (Paulitz and Bélanger, 2001). Crops have a high values and when there are limited numbers of registered fungicides this will result in a unique niche of using biocontrol agents to control plant diseases.

The use of Biocontrol agents is an increasing research area (Paulitz and Bélanger, 2001). For treatment of aerial plant diseases, the Biocontrol agents need specific environmental conditions (Romero *et al.*, 2007). High relative humidity is one example of environmental conditions that optimizes the activity of the Biocontrol agent. Efficacy may also differ between different seasons and cultivars (Dik *et al.*, 1998). Romero *et al.*, (2007) also stated that Biocontrol agents perform better under greenhouse conditions than under field conditions. Furthermore, the climate conditions in the greenhouse can affect the efficacy of the Biocontrol agent (Dik *et al.*, 1998).

Most of the Biocontrol agents require above 70% relative humidity (Dik *et al.*, 1998). The most explored agents for biocontrol of powdery mildew in cucumber are the mycoparasites *Ampelomyces quisqualis* Ces. and *Lecanicillum lecanii* (Zimm.) Zare and Gams (Romero *et al.*, 2007). Commonly when using mycoparasites, a certain level of disease has to be tolerated by the plant, because mycoparasites can only attack an already established infection (Kiss, 2003). Mycoparasites cannot usually stop the spread of powdery mildew, but it can follow the spread and reduce the damages in the infected plants (Kiss, 2003).

One of the best known mechanisms of fungal antagonism is mycoparasitism of powdery mildew by *Ampelomyces* spp (Kiss, 2003). The *Ampelomyces* spp. hyphae penetrate the powdery mildew hyphae and continue their growth inside the hyphae. The attacked powdery mildew mycelia are averted in its sporulation by the intracellular mycoparasitism (Kiss, 2003). *A. quisqualis* occurs naturally and requires free water to infect powdery mildew colonies (Falk *et al*, 1995). *A. quisqualis* is a hyperparasite and host by different powdery mildews e.g. cucurbit powdery mildew (*Golovinomyces cichoracearum* and *Podosphaera xanthii*), apple powdery mildew (*Podosphaera leucotricha* (Ell. And Ev.) Salm.), grapevine powdery mildew (*Uncinula necator* (Schw.) Burr.) and rose powdery mildew (*Spaerotheca pannosa* (Wallr: Fr.) Lév).

Pythium spp. is a broadly operative genus of oomycetes and has more than 200 described species pathogenic or saprophytic on plants, mammals and fish (Al-Sa'di *et al*, 2007). *Pythiumoligandrum* Drechs. has shown antagonistic activities in several species of pathogenic fungi (Mohammed *et al*, 2006). This antagonist produces oligandrin, which is a plant defence elicitor. In the rhizosphere, *P. oligandrum* can indirectly affect pathogen control (Kaewchai *et al*, 2009). The fungus can also induce plant resistance and induces plants to respond more rapidly and efficiently to pathogen infections. To reduce damping-off diseases *P.oligandrum* oospores have been used as seed treatment (Kaewchai *et al*, 2009).

2.3.3 Physical control

Physical control agents are for example vegetable oils and soap (Borg Ohlsson and Jansson, 2011). Oils and soaps are commonly used to control insects but can also be used to control powdery mildews. Both oils and soaps have contact action, which means that the fungi will directly be affected only if it comes in contact with the solution. In Sweden there are special 14 oil agents available, like Zence 40 (fatty acid potassium salt), Bioglans (paraffin oil) and Reniderm (fatty acid potassium salt). Common soap from the supermarket can also be used. In-house oil suspensions can be made from cold-form rapeseed oil. An alginate, Agri-50E, have been shown to have effect on powdery mildews (Borg Ohlsson and Jansson, 2011). Du *et al.*, (2010) tested the control effect of different vegetable oils on cumin powdery mildew e.g. soybean oil, sun flower oil and corn oil in both indoor protective test and field efficacy tests. They were able to show that they had a significant controlling effect on cumin powdery mildews compared to a fungicide containing triadimefon. The oils used in the experiment had a protective effect between 87.2 and 100 % and a control effect between 59.0 and 69.7 % (Du *et al.*, 2010).

2.3.4 Chemical control

Fungicides such as Carbendazim/Tridemorph (0.15%) are applied as spray solution at a dose of 400-500 l/ha. Sulphur is Man's oldest fungicide. To date, no fungicide resistance has developed despite centuries of use Sulphur is cheaper than most other fungicides and works well if sprayed well. It is best used at the high rate 25kg/ha. It has a significant advantage both direct contact and volatile action. Because powdery grows on the surface of cumin, it is killed readily by any contact fungicide active against the pathogen. As a result, sulphur has post- and pre-infection activity; that is, it both kills the pathogen (post-infection activity) and protects the new foliage (Emmett *et al*., 1994).

2.4 Other Non-Fungicide Products

Non-fungicide products can induce resistance in powdery mildew infected plants (Kiss, 2003). They can also act as prophylactic or curative factors. This commonly has an effect in greenhouse production. Common non-fungicide products are soluble silicon, milk enzyme, garlic extracts, argel extracts and sodium bicarbonate (Borg Ohlsson and Jansson, 2011).

2.4.1 Silicon Treatments

Can be used as growth improvement, mixed in the plant nutrient solution and can increase the resistance to powdery mildews in cucumber (Borg Ohlsson and Jansson, 2011). Shuerger and Hammer, (2003) and Liang *et al*, (2005) showed in their experiments that silicon can suppress powdery mildew (*Podosphaera xanthii*) in cucumber plants. Enzicur is a relatively new non-chemical method to control powdery mildew using milk enzymes (Borg Ohlsson and Jansson, 2011). It has shown good results and can be used by exemption in Sweden. Enzicur will not imply any risk for resistance problems (Dahlqvist, 2008).

2.4.2 Argel

2.4.2.1 Classification

Kingdom: Plant

Class: Magnoliopsida

Order: Gentianles

Family: Asclepiadaceae

Genus: *Solenostemma*

Species: S. argel (Del)

Argel *Solenostemma argel* Del is an aromatic and medicinal plant that contains a variety of chemically active substances (Shayoub, 2003). It is an erect perennial shrub that reaches up to 1.5-2 feet in height with numerous branches carrying opposite decussate leaves. The leaves are lanceolate to oblong ovate, with acute or sub-acute apex and cuneate base. The leaf petiole is thick. Fruit are solitary follicles, thick, ovoid, lanceolate, acuminate at the apex and they are very hard with dark purple color. Seeds are turgid, ovoid and they are channel down at one face; they are minutely tuberculate bearing an apical tuft hair (El kamali, 1991).

It is widely distributed in Egypt, Libya, Chad, Algeria, Saudi Arabia, Palestine, and Central and Northern part of the Sudan (Ahmed, 2004). However, among these above mentioned countries, Sudan is regarded as the richest source of the Argel plant which grows naturally in the northern of the Sudan and extends from Berber to Abu-Hamad, especially in the Rubatab area. In other Arabic countries and Sudan, the tradition name of this plant is Hargel. The part used of the plant is dried leaves and stems (El- Kamali, 1991).

Phyto-chemicals of medicinal properties from argel shoots had been reported by many workers (Roos *et al*., 1980; Kamel *et al*., 2000; Hamed, 2001). Suleiman *et al.*, (2009) reported that the aqueous extracts of argel have antifungal and antibacterial properties. The farmers in Kassala State put Argel shoots in porous jute sacks in the irrigation canals to be leached by water. The water was effective in controlling aphids and white flies in summer tomatoes and Egyptian bull worm in okra respectively (Elkamali and Khalid, 1996).

The pharmacological actives of different extracts of *S. argel* have been investigated by Roots *et al.,* (1980) Abdelwahab (2002) and Tharib, *et al*., (1986) which confirmed the presence of antibiotic substances in the ethanol extracts of the Argel plant as well some antifungal properties of the plant. They further confirmed additional substances or compounds isolated from the Argel stem, and were demonstrated to have antibacterial properties against both gram – positive and gram – negative organisms.

2.4.3 Usage of Baking Soda in Disease Control

Baking soda (sodium bicarbonate) is non-toxic, readily available, and very inexpensive. It can be effective against powdery mildew and somewhat useful against black spot. If you repeatedly spray leaves with bicarbonate, though, it will eventually reach the soil below, where it can accumulate and lead to slower plant growth. The salt of baking soda (sodium bicarbonate, $NaHCO3$) and potassium bicarbonate ($KHCO3$) which have been suggested for controlling various fungal diseases in plants. Similarly they were used to reduce the incidence of infection by powdery mildew in cumin crops (Reuveni *et al*., 1996; Karabulut *et al.,* 2003; Janisiewicz and Peterson, 2005 and Smilanick *et al.,* 2006).

By far the most studied application of sodium bicarbonate has been as a deterrent to several genera of foliar powdery mildew. Low levels of SBC (0.5-2%) were found to reduce germination and growth of several mildew species in the laboratory. In greenhouses, where powdery mildews are prevalent on plants of agricultural and ornamental importance, weak solutions of SBC (0.5-1.0%) have been used successfully without causing significant phytotoxicity. In field research, however, SBC has provided fewer consistent results, with good, marginal, or poor control of powdery mildew species reported equally on crops (Master Gardener magazine 2008). Low concentrations of SBC, combined with horticultural oil, may have some effectiveness on mild cases of powdery mildew.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Experimental site

This study was conducted at Dongula Research Station farm of the Agricultural Research Corporation (ARC), in Northern state during season 2014-2015. It was intended to investigate the bioactivity of crude aqueous extracts of Argel, Sodium bicarbonate and efficacy of fungicide (Jiamyl 50 WP), against *Erysiphe polygoni* the causal organism of powdery mildew in cumin and on yield of this crop.

3.2. Collection of botanical and chemicals materials

The seeds of cumin were purchased from local market of Dongula. Samples of Argel (*Solenostemma argel*) leaves were also obtained from local market of Dongula during 2015. The leaves were freed from foreign materials like stones, sand and dust. Sodium bicarbonate ($NaHCo_3$) was obtained from soil laboratory-Dongula Research station and the fungicide was purchased from pesticides shops at Khartoum North.

3.3 Layout of the Experiment, Land Preparation and sawing

The experiment was arranged Randomized Complete Blok Design RCBD replicated three times with plot size 5m×2.5m. The seeds of local variety were used in this experiment. Five treatments, each of two concentrations except fungicide which was used as recommended by ARC (Argel, Sodium bicarbonate ($NaHCo_3$) and Fungicide Jiamyl (50 WP) were applied plus untreated control. The field was prepared according to adopted method by ARC.

The seeds were sown in 20th November, five gram seeds per line, space between lines were 20cm and irrigation was given immediately after seed sowing. Irrigation was then applied every 15 days.

3.4 Application of treatments

In group one Argel was applied as foliar application (20g/L and 60g/L) after being washed with water, dried, and milled using laboratory mill into fine powder, socked in distilled water overnight and then filtered. Where in group two (Sodium bicarbonate salt), two concentrations 1.5g/L and 2g/L was used. In group three the Fungicide Jiamyl 50 WP which was used as recommended by ARC at the rate of 0.5g/L. Application of each treatment started after appearance of first symptoms of powdery mildew (60 days after sawing) and was done twice at 7 days interval.

The foliar application was achieved by knapsack sprayers. The first reading of disease incidence was taken after seven days from the first spray (31January 2015). Plastic sac was used during spraying to prevent drift of chemical by wind.

3.5 Data collection

Eight plants were labeled randomly from each subplot for recording the incidence of powdery mildew symptoms and severity in each of the three replicates. The observation of disease incidence was done on daily basis using with the help of slide microscope.

3.6 Disease incidence of powdery mildew:

Number of plants diseased out of total number of plants observed was recorded on weekly basis and expressed as percentage (%) of infected plants from total number of plants inspected as follows: -

$$\frac{\boldsymbol{No. of\ diseased\ plants}}{\boldsymbol{total\ No. of\ plants}} \times \mathbf{100}$$

3.7 Disease Severity Rating:

The amount of plant tissue affected by disease was also recorded. Disease severity was evaluated after spray by hand lens to estimating the percentage of leaf area covered by powdery mildew. Symptoms development was evaluated according to the following scale as described by (Suad *et al*., 2013).

- 0 = No visible disease symptoms (0%)
- 1 = percent of the leaf area affected (1-20%)
- 2 = percent of the leaf area affected (21-30%)
- 3 = percent of the leaf area affected (31-40%)
- 4 = percent of the leaf area affected (41-50%)
- 5 = percent of the leaf area affected (more than 50%)

3.8 Harvesting

The harvesting was performed manually.

3.9 Statistical Analysis

The collected data were subjected to standard statistical analysis. The procedure of analysis of variance and mean separation were followed according to the description of Gomez and Gomez, (1984). The data was analyzed using MSTAT-C Statistical Package.

CHAPTER FOUR

RESULT

4.1 Effect of disease Incidence after seven days from first spray.

The results (Table 1 and Figure 1) showed that spraying of all concentrations of Argel aqueous extract, NaHCO3 solution, at all concentrations and fungicide were highly effective in reducing incidence of Powdery Mildew disease after seven days from first spray compared to control.

The fungicide at 0.5g/L significantly decreased the incidence percentage of the disease which is the highest reduction in disease incidence (31%) followed in descending order by Argel (33.3%) at 60g/L, NaHCO3 (35%), in 2g and 41.7% at 1.5g/L concentration. Meanwhile, Argel at 20g/L displayed the lowest reduction in disease incidence (45%) compared to control. However, Argel aqueous extract and NaHCO3 solution exhibited an increasing efficacy with increased concentration.

Table 1 Effect of disease Incidence after seven days from first spray.

Treatments and concentration g/l		Incidence (%)
Argel	60	(33.3)5.8d
Argel	20	(45.0)6.7ab
$NaHCo_3$	1.5	(41.7)6.5bc
$NaHCo_3$	2.0	(35.0)6.0d
Jiamyl	0.5	(31.7)5.7d
Control	-	(53.3)7.3a
LSD	0.59	
SE±	0.19	
CV%	5.20	

Means followed by the same letter are not significant different at ($P< 0.05$). Data in the parentheses transformed using square root transformation $\sqrt{x + .5}$ before analysis.

4.2. Effect of disease Incidence after 14 days from first spray

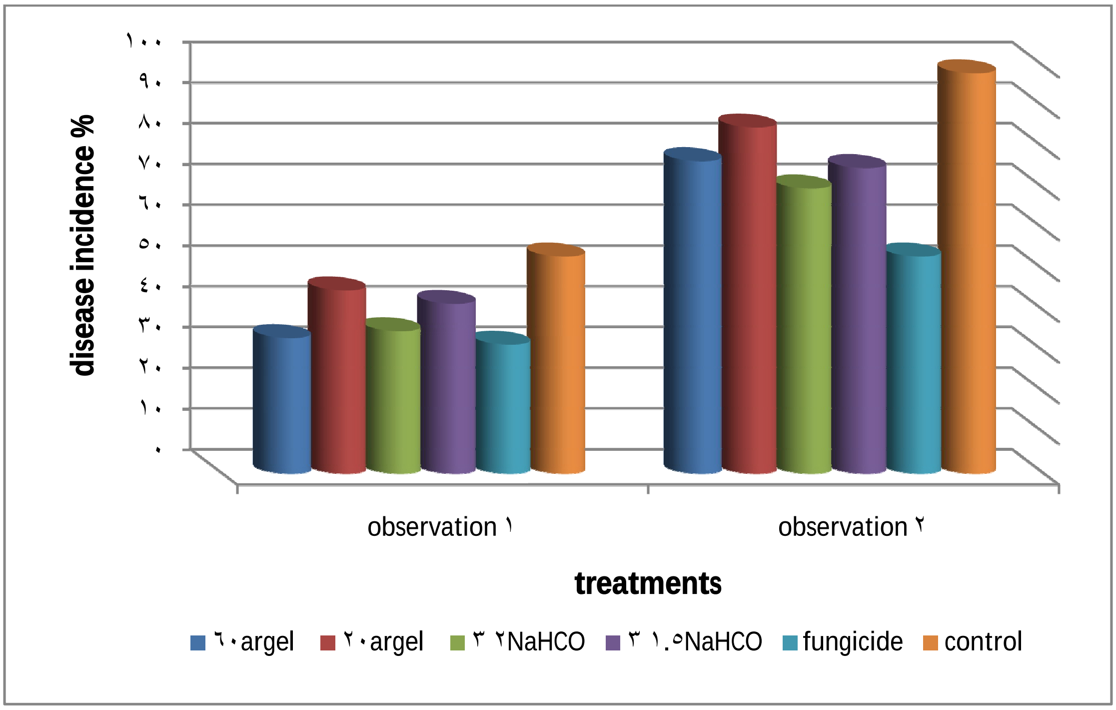
The results (Table 2 and Figure 1) showed that spraying of Argel aqueous extract, NaHCO3 salt and fungicide after seven days from second spray was also effective in reducing incidence of Powdery Mildew disease compared to control.

Likewise, the fungicide decreased the disease incidence to 53.3% which is significantly high compared to control followed in descending order by NaHCO3 in two weights 2 and 1.2g to (70.0% and 75.0%) and Argel aqueous extract in two weight 20 and 60g to (76.7% and 85.0%). However, the efficiency of NaHCO3 in controlling the disease as spray was more pronounced than Argel aqueous extract after 7 days from second spray. Likewise, the effect of both products in reducing disease incidence increased with increasing concentration.

Table 2 Effect of Disease Incidence after 14 days from first spray

Treatments and concentration g/l		Incidence (%)
Argel	60	(76.7)8.8bc
Argel	20	(85.0)9.2b
$NaHCo_3$	1.5	(75.0)8.7c
$NaHCo_3$	2.0	(70.0)8.4c
Jiamyl	0.5	(53.3)7.3d
Control	-	(98.3)9.9a
LSD		0.45
SE±		0.15
CV%		2.98 %

Means followed by the same letter are not significant different at ($P < 0.05$). Data in the parentheses transformed using square root transformation $\sqrt{x + .5}$ before analysis.



Figure, 1: Effect of disease Incidence after seven days from first spray and effect if disease Incidence after 14 days from first spray

4.3. Effect of disease severity after seven days from first spray.

The results revealed that all treatments (Argel leaves extract, $NaHCO_3$ solution and fungicide) reduced the severity of disease seven days after spraying compared to the control (Table, 3 and fig. 2). The reduction in disease severity was significantly high in all treatments compared to the control. However, the lowest severity (0.8%) was obtained by fungicide followed by $NaHCO_3$ in two weights 2 and 1.5g (1.1% and 1.3%) and Argel in two weight 60 and 20g (1.5% and 1.6%) respectively compared to control (2.3%). Similarly, the severity of the disease decreased with increasing concentrations of Argel and $NaHCO_3$.

Table 3 Effect of disease severity after seven days from first spray.

Treatments and concentration g/l			Severity scale
Argel	60		1.5b
Argel	20		1.6b
$NaHco_3$	1.5		1.3c
$NaHco_3$	2.0		1.1d
Jiamyl	0.5		0.8e
Control	-		2.3a
LSD		0.19	
SE±		0.06	
CV%		7. 59	

Means followed by the same letter are not significant different at (P< 0.05).

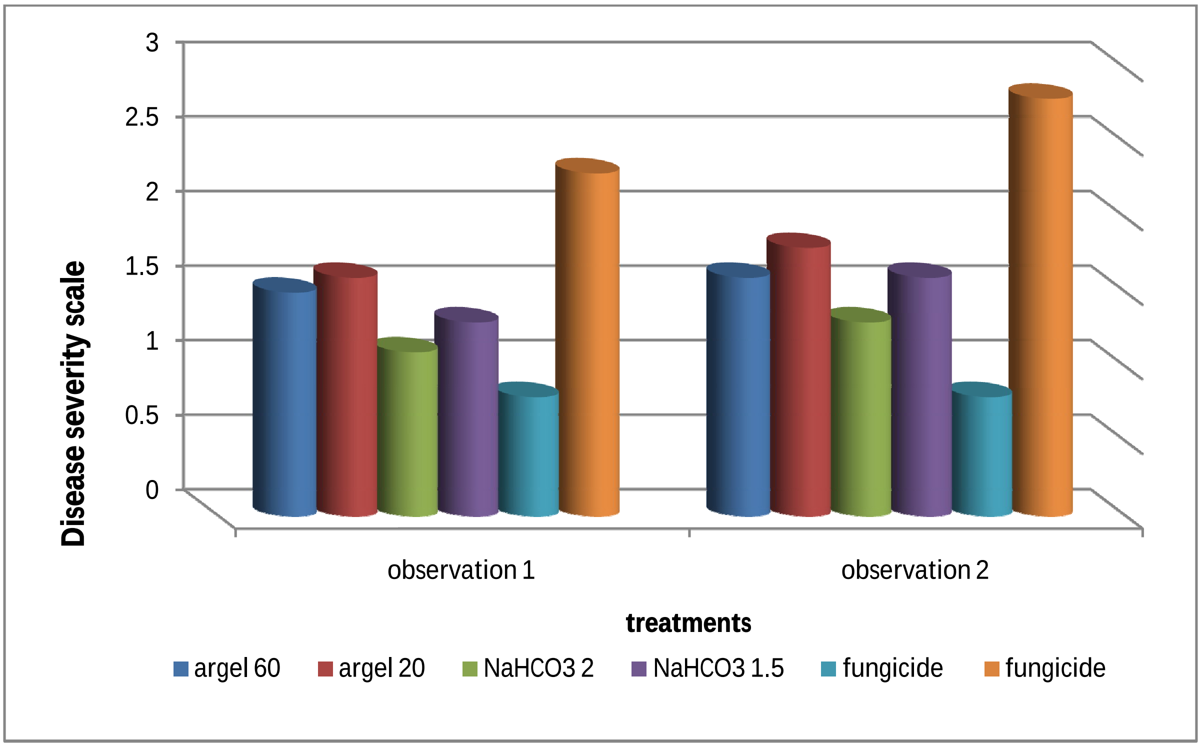
4.4. Effect of disease severity after 14 days from first spray.

Likewise, after seven days from second spray, the level of disease severity was significantly reduced by all treatments compared to control (Table, 4 and fig 2). Moreover, the efficacy of fungicide in reducing disease severity (0.8%) was more pronounced than other treatments where NaHCO3 in two weights 2 and 1.5g results in 1.3% and 1.6% which was relatively more effective at high concentration (2g/L) in reducing disease severity than Argel in two weights 60 and 20g that gave 1.6% and 1.8% respectively compared to control (2.8%).

Table, 4: Effect of disease severity after 14 days from first spray.

Treatments and concentrations g/l		Severity scale
Argel	60	1.6c
Argel	20	1.8b
$NaHCo_3$	1.5	1.6c
$NaHCo_3$	2.0	1.3d
Jiamyl	0.5	0.8e
Control		2.8a
LSD	0.15	
SE±	0.05	
CV%	5.38	

Means followed by the same letter are not significant different at (P< 0.05).



Figure, 2: Effect of disease severity after seven days from first spray and disease severity after 14 days from first spray.

4.5: Effect of different concentrations of aqueous extracts of Argel leaves, $NaHCo_3$ salt and fungicide on yield of cumin (Kg/fed).

Results (Table 5 and Figure 3) showed clearly that spraying of all treatments were highly effective in increasing yield of cumin over control. The increase in yield ranged from 173 Kg/f obtained by NaHCO3 at 1.5g/l to 253Kg/f resulted from spraying with fungicide compared to (130 Kg/f) in control. However, minimum disease incidence (53.0%) and severity (0.8%) (Table, 1 to 4) with the highest increase in yield 253Kg/f was found in treatments where cumin crop was sprayed by fungicide where as the lowest increase in yield (173Kg/f) was obtained by NaHCO3 at 1.5g/l. Moreover, the positive effect on yield with increasing concentrations of Argel and NaHCO3 was also demonstrated.

Table 5 Effect of different concentrations of aqueous extracts of Argel leaves, $NaHCo_3$ salt and fungicide on yield of cumin (Kg/fed).

Treatments and concentrations g/l		Yield (kg/ fed)
Argel	60	186.4bc
Argel	20	177.0bc
$NaHco_3$	1.5	173.9bc
$NaHco_3$	2.0	198.7ab
Jiamyl	5.0	253.5a
Control		130.7c
LSD	59.09	
SE±	19.18	
CV%	17.79	

Means followed by the same letter are not significant different at ($P< 0.05$).

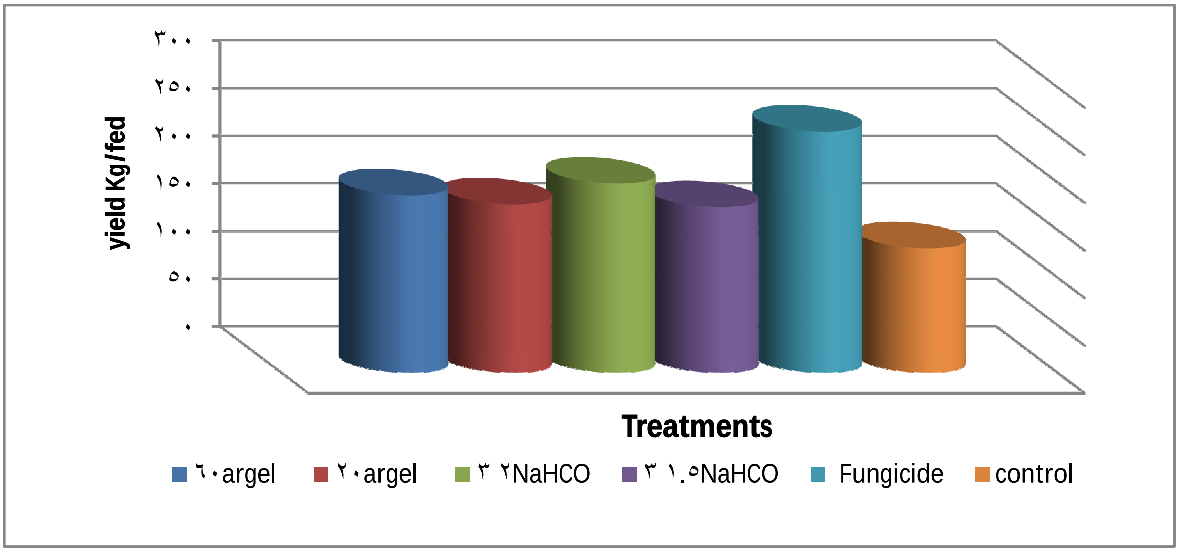


Figure 2 Effect of different concentrations of aqueous extracts of Argel leaves, $NaHCo_3$ salt and fungicide on yield of cumin (Kg/fed).

CHAPTER FIVE

DISCUSSION

Green cumin (*Cuminum cyminum* L.), is a herbaceous and medicinal crop and one of the oldest and popular seed spice worldwide after black pepper (Agarwal, 1996). In fact, it is one of the most important crops in terms of exports, income, water use efficiency and reclamation of the arid and semiarid regions (Tuncturk and Tuncturk, 2006). The major constraints facing the production of cumin worldwide are losses caused by diseases, insects, and weeds. Among these, cumin powdery mildew is considered one of the most important limiting factors for cumin production worldwide (Jansen, 1981; Sharma and Khan, 1991; Abd-El-Kareem *et al*., 2004 and PÉREZ-GARCÍA *et al*., 2009).

In Sudan, cumin is a medicinal plant that is used in food industries, drinks, cosmetics, soap, perfume and cheese (Hajlaoui, *et al*., 2010). The cultivation of the crop is widely spreading specially in the Northern parts of Sudan. Despite the relative importance of this medicinal plant in Sudan and its increasing cultivation especially in the Northern part of the county, it has not been adequately studied and very few or no attention was given by research to the area of plant protection from diseases especially powdery mildew incited by *Erysiphe polygoni* to which the crop is vulnerable (Omer *et al*., 1997).

Disease control should preferably be both efficient and environmentally friendly, which is not easy to achieve at all times. Worldwide, the chemical fungicides have been used as the main strategy for control of powdery mildew disease and subsequently increases yield production (Keinath and DuBose, 2004). Unfortunately, the indiscriminate use of the fungicides posed a serious threat to human health, environment and production of fungicide resistant pathogen strains (McGrath, 1991 and Fernandez- Aparicio *et al.*, 2009). On the other hand many higher plants extracts or products have proven to be as potent as many conventional synthetic pesticides and are effective at very low concentrations and safe (Siddig, 1993;, Abdel Moneim *et al*, 2009, Elkamali and Khalid, 1996; Kamel *et al*., 2000; Hamed, 2001, Ahmed, 2004; Idris *et al.*, 2006; Sulieman *et al*., 2009 and Aziza, 2013).

The present study which was conducted at Dongula Research Station farm of the Agricultural Research Corporation (ARC), in Northern state during season 2014-2015, aimed to investigate the bioactivity of crude aqueous extracts of Argel, Sodium bicarbonate and efficacy of fungicide (Jiamyl 50 WP), against powdery mildew disease in cumin incited by Erysiphe polygoni and on yield of this crop.

The results of this study (Tables 1-5 and Figures 1-3) revealed that the spraying of aqueous plant extracts of Argel aqueous extracts at all concentrations under field conditions were invariably highly effective in reducing incidence and severity of Powdery Mildew disease and increased the yield of the cumin crop compared to control. Similar studies which explored the Phytochemicals and repellent properties from Argel had been reported by many workers (Elhady *et al*., 1994; Kamel *et a*l., 2000 and Hamed, 2001). The increase in yield and enhancement of flowering of dry date cultivar due to application of Argel was also demonstrated by (Idris *et al*., 2011) which was attributed to either phytochemicals or growth promoting ingredients.

Moreover, plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under field and laboratory conditions (Kamel *et al*., 2000; Hamed, 2001, Ahmed, 2004; Idris *et al.*, 2006; Sulieman *et al*., 2009 and Aziza, 2013).

In this study also the efficacy of NaHCO3 soution in reducing the incidence and severity of powdery mildew disease, and in increasing the yield of cumin crop, was demonstrated experimentally under field condition (Tables 1-5 and Figures 1-3). The results showed that spraying of $NaHCO_3$ solution significantly ($p<0.05$) suppressed the incidence and severity of powdery mildew disease and increased the yield of cumin crop. These results were in line with that obtained by Reuveni *et al*., (1996) Karabulut *et al*., (2003) Janisiewicz and Peterson, (2005) and Smilanick *et al*., (2006) where NaHCO3 was used to reduce the incidence of infection by powdery mildew in cumin crops.

Obviously, there was a considerable interest in the use of sodium bicarbonate (NaHCO3) for controlling various fungal diseases in plants (Karabulut *et al*., 2003; Smilanick *et al*., 2006). Similar results were obtained by spraying plants with NaHCO3 solution and provided good control of several plant diseases (Horst *et al*., 1992; Fallik *et al*., 1996; Arimoto *et al*., 1997; Palmer *et al*., 1997; Smilanick *et al*., 1999; Janisiewicz and Peterson, 2005 and Smilanick *et al*., 2006). However, Fallik *et al*., (1997) reported that the inhibitory effect of sodium bicarbonate on microorganisms may be due to reduction of cell turgor that causes a collapse and shrinkage of hyphae and spores, resulting in fungistasis.

The data of the present study (Tables, 1-5 and Figures 1-3) also revealed that the spraying of chemical fungicide at 2.0g/l consistently and throughout the course of the experiment significantly ($p<0.05$) reduced the incidence and severity of powdery mildew disease and increased the yield of the cumin crop compared to control. These finding are in line with the findings reported by Abdelgader, (2005) on efficacy of fungicides against fungi where he found that the fungicide induced 100% inhibition against fungal growth when applied at 100ppm after 7days of exposure. Similar finding were also revealed by Mohammed, (2005) who found that when fungicides applied at 10ppm against fungi induced 100% inhibition after 4 days. However, the use of chemicals has helped to increase yields obtained (Ali, 1996), but one of the major problems with the constant use of chemicals is that resistance which can be induced in target organisms in addition to contamination of the environment with very toxic substances (Carvalho, 2004 and Okigbo, 2004). This has initiated the exploration of safe alternate products.

The data (Tables, 1-5) also demonstrated that the incidence and severity of powdery mildew disease decreased with increasing concentration of Argel aqueous extract and NaHCO3 solution. These results confirm previous studies which indicated that increasing concentrations of sodium bicarbonate resulted in a corresponding increase in efficacy (Mlikota and Smilanick 1998, 2001). Similarly, this variability in response to concentrations of Argel which expressed by reduction of incidence and severity of the disease due to increased concentrations of extracts was also reported by Aiyelaagbe, (2001). In his investigation, the author explained that the majority of the studies involving plant parts or extracts demonstrated their suppressing effects on infectious or harmful microorganisms at variable degree.

CONCLUSIONS

- Spraying of the crude aqueous extracts of Argel at all concentrations under field condition were invariably highly effective in reducing incidence and severity of Powdery Mildew disease and increased the yield of the cumin crop compared to control.

- The efficacy of $NaHCO_3$ solution in reducing the incidence and severity of powdery mildew disease, and in increasing the yield was demonstrated experimentally under field condition where spraying of $NaHCO_3$ significantly suppressed the incidence and severity of powdery mildew disease and increased the yield of cumin crop.

- Application of chemical fungicide (Jiamyle 50 WP) at 0.5g/l consistently and throughout the course of the experiment significantly ($p<0.05$) reduced the incidence and severity of powdery mildew disease and increased the yield of the cumin crop compared to control.

- The incidence and severity of powdery mildew disease decreased with increasing concentration of Argel aqueous extract and $NaHCO_3$ solution.

RECOMMENDATIONS

Based on the foregoing results the following studies were recommended;

- To further investigate the antimicrobial properties of Argel against targets organism to determine their potentials as active ingredients pesticides,

- Study was needed to optimize the time of application of bicarbonates and the concentration for controlling plant diseases.

- Further research may be needed to investigate combined application of botanical extracts and synthetic fungicide effective control of Powdery mildew diseases
- The variability in response which expressed by different disease control level towards the different concentrations of Argel aqueous extract and NaHCO3 solution could be investigated to adjust an optimum dose for controlling Powdery Mildew.

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APPENDIXS

Appendix 1

Table 1: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	5.718	1.144	10.502	0.0005
Within	12	1.307	0.109		
Total	17	7.024			

Coefficient of Variation = 5.20%

Appendix 2

Table 2: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	11.583	2.317	34.179	0.0000
Within	12	0.813	0.068		
Total	17	12.396			

Coefficient of Variation = 2.98%

Appendix 3

Table 3: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	4.071	0.814	69.790	0.0000

Within	12	0.140	0.012		
Total	17	4.211			

Coefficient of Variation = 7.59%

Appendix 4

Table 4: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	6.769	1.354	174.071	0.0000
Within	12	0.093	0.008		
Total	17	6.863			

Coefficient of Variation = 5.38%

Appendix 5

Table 5: Analysis of variance table (One way ANOVA table):

	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob.
Between	5	24052.781	4810.556	4.361	0.0171
Within	12	13237.687	1103.141		
Total	17	37290.468			

Coefficient of Variation = 17.79%

Appendix 6

Fungicide

Common Name: Benomyl

Company: jiahua crop chemical Co., China.

Agent: Sabico investment Co., Khartoum.

The recommended Dose: 0.5 g/L (0.25 g a.i. /L) .

The pest: Powdery mildew.

The Scientists in Charge: S.A. Gameil and S.M. Adam

The year of Recommendation: 2007.

Appendix 7

Plate 1

Plate 1: Effect of powdery mildew diseases on Green Cumin (control)

Appendix 8

Plate 2

Plate 2: experiment layout

Appendix 9

Plate 3

Plate 3: Green cumin growth

Appendix 10

Plate 4

plate 4: Marker 20 cm

Appendix 11

Plate 5

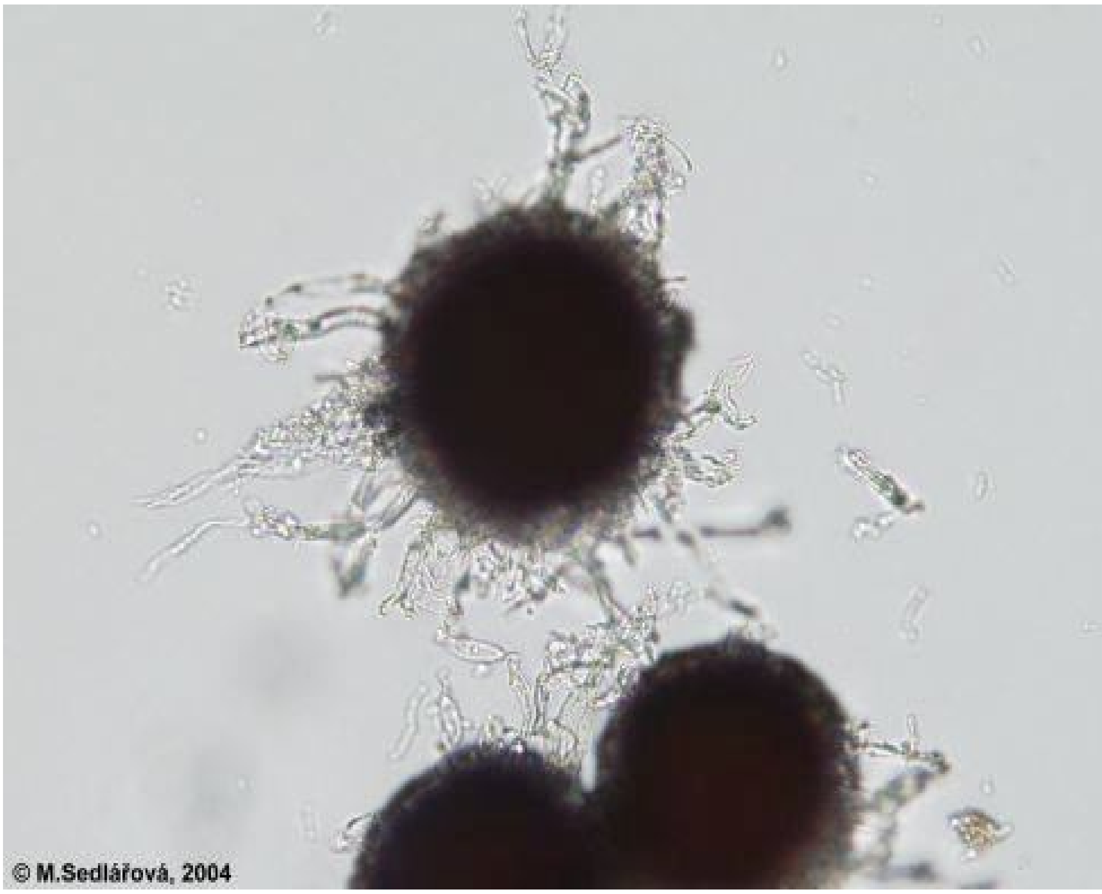

Plate 5: Fruiting structure of *Erysiphy polygoni*