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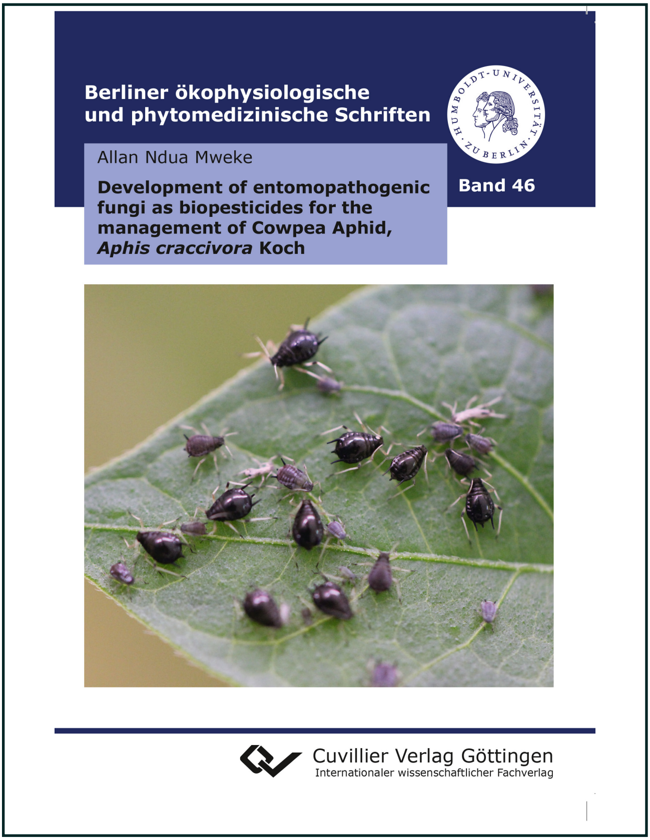
Development of entomopathogenic fungi as biopesticides for the management of Cowpea Aphid, Aphis craccivora Koch



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1.0 GENERAL INTRODUCTION

1.1 Background

Cowpea, *Vigna unguiculata* (L.) Walp (Fabaceae) is an important leguminous crop in semi-arid tropics and is well adapted to drought (Akibonde and Maredia, 2011; Horn et al., 2016; Yadav et al., 2017).

Estimated annual production of cowpea is 6.2 million metric tons (MT) occupying 14.5 million hectares of land in more than 45 countries across the world (Abate et al., 2012). Africa is the leading producer of cowpea with Nigeria, Niger and Burkina Faso accounting for 80% of the world's production (FAO, 2016). Kenya produces about 2% of the total world production mainly in the drier regions (HCD, 2014; FAO, 2016).

Consumption of cowpea either as leaves, immature pods, green peas and dry grains provides local populations affordable sources of proteins, essential vitamins and minerals (Ghaly and Alkoaik, 2010; Hall, 2012). Cowpea is used mainly as grain legume in West Africa and as a leafy vegetable in East Africa (Chiulele et al., 2011; Hall, 2012; Rusike et al., 2013). Leafy cowpea is an important African indigenous vegetable (AIV) in Kenya that is widely grown and consumed in urban and rural areas (Abukutsa-Onyango, 2010; Rusike et al., 2013). Cowpea also has medicinal benefits as its consumption has been shown to reduce environmental enteric dysfunction (Trehan et al., 2015).

The vegetative parts of cowpea plant are fed to livestock as fodder (haulms) and farmers earn income from selling them in the dry season (Rusike et al., 2013). The spreading cowpea varieties protect the soil against soil erosion and help in weed control (Garko et al., 2016). The crop improves soil fertility and soil structure by providing organic matter from crop residues and fixing atmospheric nitrogen into the soil through symbiosis with nodule forming bacteria such as *Bradyrhizobium* spp. (Mucheru-Muna, 2010; Schipanski and Drinkwater, 2012; Dwivedi et al., 2015).

1.2 Problem statement and justification of the study

In Africa, yields of cowpea are low, ranging between 100 and 250 kg/ha (Omongo et al., 1997; Baidoo et al., 2012) compared to potential yields of 3000 kg/ha in United States of America (Rusoke and Rubaihayo, 1994; Hall, 2012). Arthropod pests and diseases as well as of use of inferior varieties and/or farmer saved seeds and poor soil fertility are the key limiting factors in cowpea production (Obopile, 2006; Dugje et al., 2009). Cowpea aphid, *Aphis craccivora* Koch (Aphididae), is a major pest of cowpea that attacks the crop in all the stages of its growth (Blackman and Eastop 2006; Kusi et al., 2010; Souleymane et al., 2013). The pest contributes to yield losses of up to 100% through disruption of plant physiological growth by sacking the plant sap and slowing of photosynthetic process by reducing leaf surface area exposed to light by depositing honeydew on the leaves (Sorensen, 2009). Additional yield losses are also due to transmission of more than 30 plant viruses including Cowpea aphid-borne mosaic virus (CABMV) (Blackman and Eastop, 2000; Smith and Boyko 2007; Damiri et al., 2013).

Management of the cowpea aphid is mainly based on use of chemical pesticides, which have been reported to be expensive and ineffective (Hassan, 2013). Furthermore, pesticides have many undesirable impacts like development of resistance due to indiscriminate use, human health concerns due to exposure during application and chemical residues on plant products, bioaccumulation in the environment and disruption of ecological services of beneficial organisms (Sánchez-Bayo, 2011; Baidoo et al., 2012; El-Heneidy et al., 2015). The negative environmental impacts of the synthetic pesticides have accelerated search for alternative crop protection products leading to increased development of less toxic compounds based on naturally occurring toxins from micro-organisms (Mazid *et al.,* 2011).

Several integrated management approaches targeting cowpea aphid have been developed and used with varying degrees of success (Afun et al., 1991; Egho, 2010). These strategies have employed use of resistant varieties (Huynh et al., 2013; Smith and Chuang, 2014), use of biological control agents including the use of entomopathogenic fungi (EPF) formulated as mycoinsecticides (Hajek and Delalibera, 2010), manipulation of agro ecosystems like intercropping (Hassan, 2013), and targeted insecticide application as opposed to routine and blanket spraying (Egho and Enujeke, 2012). The plant sap sucking soft bodied aphids are susceptible to attack by EPF under natural environment and epizootics due to infection by EPF have been reported (Pell et al., 2003; Roy et al., 2010). The EPF belong to the Zygomycetes and Hyphomycetes groups but the Zygomycetes class form the larger group of EPF attacking aphids including *A. craccivora* (Humber 1991). Several isolates of *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium* spp and *Isaria* spp are commercial products currently available for use in management of several aphid species. For example, *B. bassiana* (registered as BotaniGard® and Naturalis-L®), *M. anisopliae* (registered as Met52®), *Isaria javanica* (Frieder and Bally), (Samson and Hywel-Jones), (registered as Preferal®) and *Lecanicillium* spp. (registered as Vertalec®) are all used for aphid control in Europe and North America (Zimmerman, 1992; Cook et al., 1996; Whipps, 1997; Fravel et al., 1998; Wraight and Carruthers, 1999; Copping and Menn, 2000; Hynes and Boyetchko, 2006; Jandricic et al., 2014).

Fungal based biopesticides are being used as commercial crop protection products for management of aphids in Asian, Latin American as well as and European countries which account for the greatest market share of these products with Africa registering and using the lowest percentage (Faria and Wraight, 2007). Biopesticides are increasingly becoming viable alternatives for control of insect pests due to their safety to users, non-target beneficial arthropods and environment and their compatibility with IPM strategies.

Though there are previous studies on pathogenicity of *M. anisopliae*, *B. bassiana* and *Isaria* spp on *A. craccivora* and other aphid species (Ekesi et al., 2000, Sahayaraj and Borgio 2010, Saranya et al., 2010; Bayissa et al., 2016), the studies did not evaluate the promising isolate in this study; ICIPE 62 against *A. craccivora*. Additionally, ICIPE 62 has been used to control other aphid species in vegetables and is commercially available as a biopesticide in Kenya. Moreover, none of the isolates evaluated in this study have been tested for pathogenicity against *A. craccivora* before. In Kenya, there is an EPF based biopesticide (Met 62®-*M. anisopliae* isolate ICIPE 62) developed by the International Centre of Insect Physiology and Ecology (*icipe*) in collaboration with Real IPM Kenya against major vegetable arthropod pests including aphid species (http://www.realipm.com/). However, this product does not include *A. craccivora* as one of the target pests hence this study evaluated the pathogenicity of this isolate among others for potential development of a biopesticide that can be used in the management of this pest.

1.3 General objective

This study aimed at developing and optimizing entomopathogenic fungi as biopesticides for the management of *A. craccivora* within the context of cowpea IPM.

1.4 Specific objectives

1. Screen entomopathogenic fungi isolates for their virulence against the cowpea aphid (*A. craccivora*) and select candidate isolates that can be developed into a biopesticide and used in an IPM system
2. Evaluate different formulations of the selected isolate for the management of *A. craccivora* on cowpea under field conditions
3. Assess the efficacy of intercropping cowpea with maize and application of selected EPF isolate for the management of *A. craccivora*.

1.5 Research questions

This study set out to answer the following questions:

4. Does pathogenicity of entomopathogenic fungi to *Aphis craccivora* vary within and among isolates of different species?
5. Is production of fungal spore on insect cadavers positively related to isolate(s) virulence?
6. Does formulation type influence performance of entomopathogenic isolates both in screenhouse and field conditions?

7. Is combination of intercropping cowpea and maize and application of entomopathogenic fungi more effective in suppressing aphid population under field conditions compared to application of Duduthrin or non-application of either EPF or Duduthrin in a cowpea maize intercrop?

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2.0 SCIENTIFIC BACKGROUND

2.1 *Vigna unguiculata* L. Walp

Origin: Cowpea (*Vigna unguiculata* L. Walp.) is an annual drought tolerant legume that is adapted to different soil types and different cropping systems whose origin has been traced to Southern Africa region (Singh et al., 1997). The West African countries including Nigeria, Niger, Burkina Faso, Benin, Togo and Cameroon have the biggest diversity of cultivated cowpea (Ng and Marechal; 1985; Ng, 1995; Padulosi and Ng, 1997; Timko and Singh, 2008).

Global production*:* Cowpea (*Vigna unguiculata* L. Walp).is grown mostly in tropical Africa (Ofuya, 1997) and Africa produces the largest proportion of the world's production with Nigeria, Niger and Burkina Faso producing 80% of world's production (Mortimore et al., 1997; FAO, 2016). In 2016, the world production was 6,991,174 metric tons with a total value of U$ 18 billion while the total area under production was 12.3 million hectares. In the same year Africa produced 6,739,689 metric tons representing 96% of the world production (FAO, 2016).

Production in Kenya*:* Major cowpea production localities in Kenya are largely in the arid and semi-arid areas since it is a drought tolerant crop and farmers can harvest even when cereal crops like maize and sorghum fail (Saidi et al. 2010). In 2016, the total area under production was 31,020 hectares producing 115,801tons valued at Kenyan shillings 2.4 billion representing about 2% of the world production with Makueni County producing 35% of the total production (HCD, 2016). Areas of production include Machakos, Kitui, Makueni, Tharaka, Mbeere, Taita Taveta, Kwale, Kilifi, Lamu, Kisii, Migori, Homabay, Siaya, Kisumu and Bugoma The cowpea varieties grown by farmers can be categorize into 4 according to their seed colour or mode of growth (1) the cream types which have cream colored seeds, (2) the crowder type with black spots or brown colour, (3) the black eye types whose seeds are white with black eye and (4) other types with intermediate colours. Some commercial varieties in Kenya and East African region include Kunde M66, Ken Kunde 1(KK1) Ken Kunde 3(KK3), K80, KVU 27-31 and KVU 419.

Uses*:* Cowpea crop has a wide adaptability to different climatic conditions and it is cultivated in warm regions of the world mainly for its edible seed, however, the crop is also an important source of vegetable and, it is one of the most important African leafy vegetables (Hall, 2012; Rusike et al., 2013). Cowpea does well in poor soils because it has tolerance to low soil fertility and it has ability to fix atmospheric nitrogen in association with root nodule forming bacteria (*Bradyrhizobium* spp (Schipanski and Drinkwater LE 2012; Ddamulira et al., 2015) and it also able tolerate a wide range of soil pH (Mucheru-Muna, 2010).

In Kenya and other East African countries, cowpea has wide nutritional and agronomic uses. Young leaves are used as a vegetable while the seeds and young pods constitute a rich source of dietary protein. Dried shoots and roots are used as fuel (Hall, 2012; Trehan et al., 2015). Cowpea seed is a nutritious component in the human diet as it contains about 25% protein and 64% carbohydrate, and 2% fat (Akibode, 2011; Owolabi et al., 2012).The leaves have higher protein content compared to seed (Baker et al. 1989; Nielsen et al., 1993). Cowpea is widely consumed all over the world, mainly in rural populations, and satisfy a considerable proportion of the protein requirements (Oiye et al., 2009; Ghaly and Alkoaik, 2010).

Above ground parts of cowpea plant excluding the pods are used as fodder for livestock (haulms) and a source of income for farmers who harvest and sell during dry period (Singh et al., 1997). The spreading and indeterminate or semi-determinate varieties of cowpea provide ground cover against soil erosion while at the same time suppressing weeds (Singh et al., 1997; Mucheru-Muna, 2010). The cowpea crop residues when ploughed into the soil provide organic matter that improves soil fertility (Mucheru-Muna, 2010). Cowpea being a leguminous crop fixes atmospheric nitrogen into the soil through symbiosis with nodule forming bacteria (*Bradyrhizobium* spp), (Singh et al., 1997; Schipanski and Drinkwater, 2012; Dwivedi et al., 2015).

2.2 The Cowpea aphid *Aphis craccivora* (Koch)

Taxonomic description*:* Compared to other aphid species, *A. craccivora* is a relatively small aphid. Apterous females are characterized by black or dark brown body, brown to yellow legs and a prominent cauda. Nymphs are waxy compared to adults. Adults are distinguished from other closely related aphid species by the presence of 6-segmented antennae with black distal part of femur, siphunculi and cauda (Blackman and Eastop, 2006). Winged (alate) females of *A. craccivora* have distinct dorsal cross bars on the abdomen (Blackman and Eastop, 2000).

Distribution and occurrence: *Aphis craccivora* is widely distributed in the world and it has been reported in regions/countries where it was absent mainly due to changing climate but it is more endemic in the tropics (Blackman and Eastop, 2000).

Biology and ecology

Aphis craccivora has a wide distribution in the tropics where females reproduce parthenogenetically but sexual morphs have been reported in temperate regions (Blackman and Eastop, 2007). Cowpea aphid females are ovoviviparous and retain their eggs inside their bodies and give birth to nymphs. Small colonies of *A. craccivora* establish on actively growing plant parts like leaves, tips and young stems and are frequently found in association with ants (Flatt and Weisser, 2000; Espadaler et al., 2012). A number of biotypes of *A. craccivora* have identified (Ofuya, 1997; Sorensen, 2009).

Development of *A. craccivora* is influenced by climatic conditions including temperature (24-28.5°C), relative humidity 65% RH, hours of sunshine (day length-L: D 16:8) and precipitation (Mayeux, 1984). Host plant biochemistry like low levels of hydrocarbon positively influences development of alate individuals (Mayeux, 1984). The lifespan of adult's ranges between 5-15 days and under favourable weather conditions *A. craccivora* completes a generation in 10 to 20 days. An adult aphid can produce 20 nymphs in one day and developmental period between first instar and adult is between 3-5 days while a single adult female can produce up to 100 nymphs in their life time (Ofuya, 1997). Weather conditions, soil moisture content and fertility as well as host plant status influence growth, development, reproduction and the lifespan of *A. craccivora* (Ofuya 1997).

Host range

Aphis craccivora is a highly polyphagous aphid species feeding on Leguminoseae group of plants including cowpea (*Vigna unguicalata* (L.) (Walp.), groundnut (*Arachis hypogaea* L.), mungbean (*Vigna radiata* (L.) R. Wilczek), pigeonpea (*Cajanus cajan* (L.) Huth), chickpea (*Cicer arietinum* L.), green beans (*Vicia* spp. and *Phaseolus* spp.), lupins (*Lupinus angustifolius* L.), lentil (*Lens esculenta*) and lucerne (*Medicago sativa* L.). It is also reported as a minor pest on other leguminous non-leguminous crops, such as cotton and citrus (Blackman and Eastop, 2006; Brady and White, 2013).

Economic importance of *Aphis craccivora*

Direct crop damage: *Aphis craccivora* causes direct damage by sucking plant sap and injecting toxins into the phloem during all plant growth stages including seedlings, flowers and pods and this damage is by both adults and nymphs (Ofuya, 1997; Huynh et al., 2015). When heavy infestations occur during early plant growth stages young plants wither and eventually die and those that survive are characterized by stunted growth, distorted leaves and experience delayed flowering and lower yields (Ofuya, 1995). Heavy infestation by cowpea aphid at podding stage can reduce seed yield (Ofuya, 1997).

Indirect damage*:* High population of *A. craccivora* produces high amounts of honeydew on plant leaf surfaces thereby promoting growth of the sooty mold fungus which interferes with respiratory and photosynthesis capacity of the plant by altering biochemical and physiological processes of infested plants thereby reducing plant growth and associated yield (Gomez et al., 2004; Sorensen, 2009; Goławska et al., 2010). Honeydew also reduces quality of cowpea leaves and renders them inedible thus contributing to yield loss. *Aphis craccivora* is a known vector of more 30 plant viruses including cowpea mosaic virus, ground nut rosette virus (GRV), subterranean clover stunt virus (SCSV) (clover stunt virus), Bean common mosaic virus (BCMV) (bean mosaic virus, bean western mosaic virus, mungbean mosaic virus) (Atiri et al., 1986; Blackman and Eastop, 2000; Brault et al., 2010).