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Allelopathy in Sorghum as a Possible Tool for *Striga hermonthica* Management

التضاد في الذرة الرفيعة أداةً ممكنة لإدارة البودا

A Thesis submitted in fulfillment of requirements for the Degree of Doctor Philosophy (Ph. D) in Plant protection (Weed Science)

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Dedication

I dedicate this work to souls of my father, mother, sister Marwa and my friend Nadia Hamaza who all ways wished to see me complete my study but they never lived to see me through. I dedicate this work, to my brother, sisters and my small family.

Mashair

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Abstract

The root parasitic weed *Striga hermonthica* constitutes a major constraint to cereals production in sub-Saharan Africa. Several control measures have been recommended, however, incompatibility with the prevalent low inputs production systems precludes their adoption. Resistant genotypes could provide an ideal solution; however, durability of resistance is contestable. A series of laboratory, greenhouse and field experiments was undertaken at the College of Agricultural Studies, Sudan University of Science and Technology (Shambat) to determine i) the influence of genotypes, growth stages and assay procedure on induction of germination, radicle length and haustorial initiation in *S. hermonthica* by root exudates and shoot and root powders ii) importance of resistance and tolerance in refuting *Striga* infection and damage as influenced by *Striga* seed bank size and iii) the feasibility of employing plant residues to deplete the parasite seed bank. Three sorghum genotypes, Wad Ahmed, *Striga* tolerant improved cultivar, Tetron, *Striga* resistant local landrace and Hakika, *Striga* resistant exotic genotype, were used. Root exudates of hydroponically grown plants collected 7- 28 days after sowing (DAS) displayed differential germination inducing activity. At 7 and 14 DAS mean germination was highest (33.7-29%) for Wad Ahmed and significantly lowest for Tetron and Hakika. At 21 DAS, root exudates from Wad Ahmed and Hakika displayed comparable and significantly higher mean germination than their congener from Tetron. At 28 DAS, Tetron root exudates induced the highest mean germination (34.7%), whereas root exudates from Wad Ahmed and Hakika were significantly less active. Radicle length and haustorium initiation were influenced by sorghum genotype, exudates volume and time of root exudates collection. Striga Root exudates from Wad Ahmed sustained the shortest radicle (0.3 and 1.2µm×10^{-2}) and highest haustorium initiation

(94.2%), whereas its congeners from Hakika and Tetron sustained the longest radicle length (4.5µm×10^{-2}) and the lowest haustorium initiation (1.6 and 0.5 %). Investigations using the rhizotron technique revealed that the genotypes sustained about equal germination and attachment of the parasite. However, the parasite development on Tetron and Hakika was arrested subsequent to xylem-to-xylem connection. Irrespective of sorghum genotype, *Striga* emergence and dry weight increased with increasing seed bank size. Wad Ahmed sustained the highest *Striga* emergence and dry weight followed in descending order by Tetron and Hakika. Further, Wad Ahmed displayed the highest reductions in plant height (39.6%), relative leaf chlorophyll contents (61.7%) and shoot dry weight (89.5%), while Hakika showed the lowest. Powder from air-dried shoots collected from field growing sorghum plants at 40 DAS induced the highest (44.1-51.7%) germination while those collected at 120 DAS induced the lowest (6.8-17.1%). For roots powder, irrespective of amount and growth stage, mean germination was invariably maximal for Wad Ahmed (92.7-96.6 %) and minimal for Hakika (0.0-13.8%). *Striga* seedlings from seeds induced to germinate by sorghum shoot or root powder, irrespective of genotypes, powder amount or sampling date displayed radicle length of 0.1- 0.6 µm ×10^{-2}. Root residue assayed subsequent to harvest (DSH), irrespective of genotype, induced low to moderate germination (10-50%). Germination increased with powder amount, reached a maximum and subsequently declined. Radicle length, affected by root residues collected after harvest, was invariably short (0.0- 0.04µm ×10^{-2}) and varied with genotype and collection time. For roots residues collected 60 DSH Striga seedlings induced by powder from Wad Ahmed and Tetron displayed, significantly, longer mean radicle length (0.02µm ×10^{-2}) than those elicited by Hakika (0.01µm×10^{-2}). Mean radicle length was maximum and significantly the longest at 10 mg /well. Higher residues amount significantly suppressed

radicle length. For roots residues collected 75 DSH radicle length was the longest ($0.04\mu m \times 10^{-2}$) for seedlings induced by powder from Wad Ahmed root residues and significantly shorter ($0.03\mu m \times 10^{-2}$) for those elicited by powder from Tetron and Hakika. The study revealed that germination inducing activity of root exudates of hydroponically grown plants and that of shoots and roots powder collected from field growing plants together with their effects on radicle length and haustorium initiation were influenced by genotype, growth stage and growth medium. The differential germination inducing activity of the hydroponically and rhizotron grown plants taken in conjunction with the differential resistance observed in the green house experiment are in conformity with the reported production of hydroxylated Strigolactones (SLs) which are extremely labile in soil and water by Tetron and Hakika. Further, in addition to the reported pre-attachment resistance to *Striga* due to low stimulant production the study revealed, for the first time, that the genotypes Tetron and Hakika are endowed with post-attachment resistance which obstructs development of the parasite after establishment of xylem-to-xylem connection. The notable fluctuations of germination and radicle length with time and/or on increasing root exudates volume or shoot or root powder amount suggest changes in production and/or proportions of germination stimulants and inhibitors and radicle extension suppressors with growth stage. Due to low germination inducing activity sorghum roots residues of the tested genotypes are of no practical value for depletion of *Striga* seed bank. However, the high and persistence activity of the residues as haustorium inducers and radicle elongation suppressor deserves to be studied further as it may reduce contact between the parasite and the host roots.

مخـلــص الأطـروحــة

تشكل الحشائش الطفيلية الجذرية البودا (*Striga hermonthica*)عائقاً رئيسياً أمام إنتاج الحبوب في أفريقيا جنوب الصحراء الكبرى لذلك تمت التوصية بالعديد من تدابير الرقابة ونسبة لعدم التوافق مع أنظمة إنتاج المدخلات المنخفضة السائدة يحول دون إعتمادها. يمكن أن توفر الأنماط الجينية المقاومة حلاً مثاليًا ومع ذلك تم إجراء سلسلة من التجارب المعملية والمشتلية والحقلية بكلية الدراسات الزراعية، بجامعة السودان للعلوم والتكنولوجيا (شمبات) وذلك لتحديد 1) أثر الأصناف وأطوار النمو في تحفيز الإنبات وطول الجذير والبدء في تكوين الممصات للبودا بواسطة إفرازات الجذور ومسحوق الساق والأوراق 2) أهمية المقاومة والتحمل في دحض الإصابة بالعدوى والأضرار الناجمة عن البودا والتي تتأثر بحجم مخزون بذور البودا 3) تقييم جدوى إستخدام مخلفات النباتات للقدرة على إستنفاد مخزون بذور الطفيل في التربة. تم إستخدام ثلاثة طرز وراثية من الذرة الرفيعة هي ودأحمد الصنف المحسن المتحمّل للبودا والصنف تترون السلالة المحلية المقاومة للبودا والصنف حقيقة النمط الجيني الدخيل المقاوم للبودا. الإفرازات الجذرية للنباتات المزروعة في الماء التي تم جمعها بعد 7–28 يومًا من البذر (بعد البذر) أظهرت نشاطًا للإنبات التفاضلي. في 7 و 14 بعد البذر ، كان متوسط الإنبات أعلى (33.7–29٪) لود أحمد وأقل بشكل ملحوظ في حقيقة و تترون. في DAS 21 ، أظهرت إفرازات الجذر من ود أحمد و حقيقة متوسط إنبات مماثل وأعلى بكثير من نظائرها من تترون. في 28 بعد البذر ، تسببت إفرازات جذر لتيترون في أعلى متوسط إنبات (34.7٪) ، بينما كانت إفرازات الجذر من ود أحمد وحقيقة أقل نشاطًا بشكل ملحوظ. تأثر طول الجذور وبدء الممص بالنمط الوراثي للذرة الرفيعة ، وحجم الإفرازات ووقت جمع الإفرازات الجذرية. اظهر ود أحمد أقصر جذر (0.3 و 1.2 ميكرومتر × 10^{-2}) وأعلى بدء للممص(94.2 ٪)، بينما حافظت نظائرها من حقيقة و تترون على أطول طول للجذر (4.5 ميكرومتر× 10^{-2}) واقل بدء للممص(0.5–1.6٪). ظهرت الفحوصات التي أجريت باستخدام تقنية الرايزوترون أن الطرز الوراثية تحافظ على إنبات وتعلق متساويين ، ومع ذلك تم إيقاف تطور الطفيل في تترون وحقيقة بعد إنشاء إتصال نسيج الخشب بنسيج الخشب. وبغض النظر عن صنف الذرة الرفيعة زاد ظهور البودا والوزن الجاف مع زيادة حجم مخزون البذور. وحقق ودأحمد أعلى ظهور للبودا وأعلى وزن جاف وأعقبه ترتيب تنازلي كل من تترون

وحقيقة. بالإضافة إلى ذلك أظهر ود أحمد أكبر إنخفاض في الطول (39,6٪) ومعدل إنخفاض محتوي الكلورفيل (61,7٪) والوزن الجاف للساق (89,5٪)، بينما أظهرت حقيقة أقل إنخفاض. على الرغم من إختلاف الأنماط الجينية في إستجابتها للطفيل فإنها استجابت في حجم مخزون البذور المرتفع. تسبب المسحوق من الفروع المجففة بالهواء التي تم جمعها من نباتات الذرة الرفيعة التي تنمو في الحقل عند 40 يوم من الزراعة في تحقيق أعلى إنبات (44.1–51.7٪) بينما أدت تلك التي تم جمعها عند 120 يوم من الزراعة إلى أقل إنبات (6.8–17.1٪). بالنسبة لمسحوق الجذور ، بغض النظر عن الكمية ومرحلة النمو ، كان متوسط الإنبات دائمًا هو الحد الأعلي بالنسبة لود أحمد (92.7–96.6٪) اقل مايمكن بالنسبة للحقيقة (0.0–13.8٪). تظهر بادرات البودا التي نبتت عن طريق مسحوق سيقان أو جذور الذرة الرفيعة ، بغض النظر عن الأنماط الجينية أو كمية المسحوق أو تاريخ أخذ العينات ، طول الجذور من 0.1 إلى 0.6 ميكرومتر × 10^{-2}. أظهرت بقايا الجذور (بعد الحصاد) بغض النظر عن التركيب الوراثي إنبات منخفض إلى متوسط (10–50٪). زاد الإنبات مع كمية المسحوق ، ووصل إلى الحد الأقصى ثم انخفض لاحقًا. كان طول الجذر المتأثر بمخلفات الجذور التي تم جمعها بعد الحصاد قصيرًا دائمًا (0.0–0.04 ميكرومتر × 10^{-2}) ومتنوعًا باختلاف التركيب الوراثي ووقت التجميع. بالنسبة لبقايا الجذور التي جمعت بعد60 يوم من الحصاد، بادرات البودا التي حفزت من مسحوق ودأحمد وتترون أعطت أعلى متوسط طول الجذير (0.02 ميكرومتر × 10^{-2}) بصورة معنوية من تلك التي تم الحصول عليها بواسطة حقيقة (0.01ميكرومتر × 10^{-2}). كان متوسط طول الجذر الأقصى والأطول بشكل ملحوظ عند 10 ملجم / للخلية. أعلي كمية للبقايا أدت إلي خفض طول الجذير معنوياً. بالنسبة لبقايا الجذور التي تم جمعها بعد75 يوم من الحصاد (بعد الحصاد)، كان الطول الأقصي (0.04ميكرومتر × 10^{-2}) للبادرات التي تم تحفيزها بمسحوق بقايا جذور ودأحمد ومعنوياً أقصر بكثير (0.03 ميكرومتر × 10^{-2}) تلك التي تم الحصول عليها بواسطة مسحوق من تترون وحقيقة. أوضحت الدراسة أن النشاط المحفز للإنبات من مستخلص الجذور للنباتات المزروعة في الماء وتلك التي تم الحصول عليها من مسحوق السيقان والجذور والمجمعة من النباتات المزروعة في الحقل تأثيرها على طول الجذير وبدء الممصات تتأثر بالأصناف ومرحلة النمو ووسط النمو. يؤكد نشاط تحفيز الإنبات التفاضلي للنباتات المزروعة بالزراعة المائية وطريقة الرايزوترون إن إنتاج الهيدروكسيل SLs أقل استقرارًا في الإفرازات الجذرية لتترون وحقيقة. بالإضافة إلى مقاومة

الإلتصاق المسبق للبودا بسبب انخفاض إنتاج المنشطات وكشفت الدراسة لأول مرة أن الاصناف تترون وحقيقة تتمتعان بمقاومة ما بعد الإلتصاق مما يعيق نمو الطفيل بعد تكوين نسيج الخشب والاتصال بالخشب. تشير الإختلافات الملحوظة في الإنبات وطول الجذير مع مرور الوقت أو في زيادة حجم إفرازات الجذور أو كمية الفروع أو مسحوق الجذور إلى تغييرات في الإنتاج أو نسب محفزات ومثبطات الإنبات ومثبطات تمديد الجذور مع مرحلة النمو.

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Abbreviations

cm	centimeter
DAS	Days after sowing
DW	Distilled water
et al	And others
Fig	Figure
Fed	Feddan
GFFP	Glass fiber filter paper
GR24	Synthetic germination stimulant
H	Hour
i.d.	Internal diameter
Kg	Kilogram
L	Liter
mg	milligram
ml	Milliliter
Mm	Mille molar
No.	Number
N_2	Nitrogen
N_0	Zero nitrogen
N1	18.6kg=40kg urea/fed
N2	37.2kg=80kg urea/fed
PDA	Potato dextrose agar medium
PP	Page
ppm	Part per million
V/v	Volume over volume
µL	Micro liter
µM	Micro molar
%	Percent
mg /pot	milligram/pot
RLCC	Relative leaf chlorophyll content

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CHAPTER ONE

INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) is the staple food of the poor and the most food in secure people, living mainly in the semi-arid tropics. It is the fifth leading cereal crop in the world after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.) and barley (*Hordeum vulgare* L.) (Menezes *et al*, 2015) and the first in Sudan (FAO, 2016).To day, sorghum is cultivated across the world in the warmer climatic areas. In Africa, sorghum is still largely a food crop.World annual sorghum production is over 60 million tonnes, of which Africa produces about 20 million.Sorghum in Sudan is the main staple food crop especially in rural areas, that grown in both traditional and semi-mechanized rain-fed areas (El Naim *et al*., 2012). However, Sudan's sorghum productivity is low compared with other sorghum producing countries. Sorghum productivity in Sudan ranged between 104- 200 kg/ feddan (Ahmed, 2009; MAF, 2010). It represents about 14%, 15% and 16% of that of Argentina, USA and China, respectively (Karrar *et al*., 2006). In Sudan, the area under sorghum constitutes about 74% of the area under cereals and 45% of the total cultivated areas (Babiker, 2007a).

Parasitic weeds represent one of the most destructive and intractable problem to agricultural production in both developed and developing countries. About 20 families (3,000–5,000 species) of higher plants are parasitic on other plants and may cause production losses of 30–80% in staple food and industrial crops on every continent. Compared with the other weeds, parasitic weeds are difficult to control by conventional means because of their life style (Aly, 2007).

Among the parasitic angiosperms witchweeds (*Striga* spp.) and broomrapes (*Orobanche* and *phelipanche*.), in the family Orobanchaceae, are root

parasitic weeds of significant economic impact on agriculture in many countries across the globe (Babiker *et al.,* 2007).*Striga hermonthica*(Del.) Benth or the purple giant African witchweed is parasitic on maize, sorghum, rice, pearl millet [*Pennisetum glaucum* (L.) R. Br.] and Sugarcane (*Saccharum officinarum* L.). It has become an increasing problem to small-scale subsistence farmers in sub-Saharan Africa and represents, today, the largest single biological barrier to food production in the region (Ejeta, 2007). Yield losses depend on the level of *Striga* infestation, the soil nutritional status, the agro-climatic conditions, the plant species, and the genotype grown (Oswald and Ransom, 2004).

In sub-Saharan Africa, *S.hermonthica* has been estimated to affect cereal crops on over 21 million ha (Sauerborn, 1991) where farmers lose 20–80% of their yields, equivalent to 4.1 million tons of grain per year. Such considerable yield losses affect livelihoods of approximately 100 million people (Kanampiu *et al*., (2002).Infection by *Striga* spp., pending size, causes yield losses ranging from slight (5%) to complete crop failure (Gurney *et al*., 2002; Rodenburg *et al*., 2005).

In subsistence farming systems, *Striga* spp. are important and persistent cereal production constraints (Scholes and Press, 2008). The parasitic weeds occur throughout sub-Saharan Africa and negatively impact the region′s economy (Parker, 2009).Sorghum production is seriously constrained by *S. hermonthica.* Yield losses were reported to range between 65-100% and complete crop failures is not uncommon under heavy infestations (Hamdoun and Babiker, 1988). Moreover, sorghum grains represent more than 60 % of poultry diets in Sudan. The plant residues are used as livestock feed. The crop performs better under adverse soil and weather conditions as compared to other crops. At least one third of the total cropped area in Sudan is annually placed under sorghum, producing about 75 % of food grains in the country. About 93 % of the

total sorghum area is in the rain-fed sector. However, the total production varies from year to year due to the quantity and distribution of rains as compared to other crops in Sudan, the productivity is very low compared to the international standards (Karrar *et al*., 2012). Drought is an important environmental factor affecting the productivity of crops (Ahmed *et al*., 2016).

In Sudan *S. hermonthica* has been recognized as a problem of national importance since the 1940 (Babiker, 2007 b). The second National Conference on Pest Management held at the University of Gezira proclaimed *S. hermonthica*as one of four major weeds affecting agricultural production in the country with sorghum and millet being the most affected (Babiker *et al.*,2007). Nitrogen and phosphorus deficiency, as well as water stress, accentuate the severity of *Striga* damage to the hosts. *Striga* is particularly a pest on low fertile soils and usually the infection decreases if mineral nutrients, especially nitrogen and phosphorus, are applied in sufficient quantities (Adagba *et al*., 2002). Fertilizer had significant effects on height, vigor, reaction of sorghum as well as shoot count, and days to emergence, dry matter production and dry weight of *Striga.* Nitrogen at high rates (N) increases the performance of cereal crops under *Striga* infection. This is due to the fact that nitrogen reduced the severity of *Striga* attack while simultaneously increasing the host performance (Lagoke and Isah, 2010).

One of the many mechanisms by which plants overcome nutrient deficiency is to engage in symbiosis with *Arbuscular mycorrhizal* (AM) fungi (Akiyama *et al*., 2005). Under nutrient deficiency, plants activate AM fungi through underground communication by releasing signaling molecules. Upon activation by these molecules, the strigolactones, a symbiotic relationship is established in which the AM fungi facilitate mineral nutrient uptake by the host, particularly of P and N, in return for

carbohydrates (Harrison, 2005). Upon strigolactone perception, AM fungi engage in hyphal branching, aprocess that improves the host root colonization success rate. Strigolactones are hence a crucial factor in the establishment of this symbiosis, but at the same time, the germination of parasitic weed seeds is induced by these same signaling molecules (Bouwmeester *et al*., 2003; Akiyama and Hayashi, 2006; Bouwmeester *et al*., 2007). In line with the importance of AM fungi for the uptake of mineral nutrients, it has been demonstrated that strigolactone exudation is increased under mineral nutrient deficiency, particularly of P (Yoneyama *et al*., 2007a, b; Lopez-Raez *et al*., 2008). Several methods of control including agronomical, chemical and genetical were explored, however, none of these methods when used alone gives adequate and reliable control of the parasite. The need for the development of a simple cheap method of control to be deployed in an integrated management strategy is therefore imperative.

The present investigation was therefore set to study (i) the effects of sorghum, root exudates and residues on *Striga* germination, (ii) the possibility of using sorghum residues for Striga management and (iii) distribution and effects of soil placement on persistence of stimulants from sorghum residues.

CHAPTER TWO
LITERATURE REVIEW

2.1. General:-

This review comprises of Sorghum, the main host of *S. hermonthica*, economic importance, and allelopathy. Furthermore, the review also includes some ecological and biological aspects of parasitic weeds with emphasis on *Striga*.spp.

2.2. Sorghum:-

Sorghum bicolor (L.) Moench, a Poaceae, production takes places across the African continent with Nigeria, Sudan, Ethiopia and Burkina Faso accounting for nearly 70% of production. Sorghum has been domesticated since approximately 3000 years B.C. in Ethiopia region (Ayana and Bekele 1998) and parts of Congo, with secondary centers of origin in India, Sudan, and Nigeria, where it is mainly used as a human food (Berenji and Dahlbrg, 2004). In Europe, sorghum is still considered as the 'poor farmer`s crop' of Africa and Asia and very limited research efforts have been undertaken to improve the crop for European conditions (Berenji and Dahlberg, 2004).

2.2.1. Economic Importance:-

Sorghum is widespread throughout the inter-tropical zone and its cultivation now extends well into the temperate regions. It is a major grain cereal of the tropical savannah vegetation region. It is one of the world's major food crops particularly in the semi-arid tropics of Africa and India which are characterized by high temperature and low rainfall (Doggett, 1988).The crop is the main staple in Africa, the Middle East, and Asia. It provides grain for consumption in form of stiff or thin porridges, steam-cooked products such as couscous, or beverage for the resource-poor farmers. The leaves and stems are also used as forage for livestock,

building materials and fuel for cooking.In the industrialized countries (Rooney and Waniska, 2000) sorghum is generally used as animal feed. Due to its ability to grow in some of the world's most austere environments, its versatility as a food and feed grain, and its ability to produce high yields, sorghum will continue to be one of the most precious cereal commodities (Frederiksen, 1986). Sorghum is the most important crop in Sudanese economy and diet. It ranks first in total tonnage of grains and total area cultivated (Babiker, 2007). However, yields are substantially low. A whole range of growth-reducing factors is responsible for this low grain yield. The inherent low fertility of most tropical soils is to be blamed for the low yields. These soils are low in organic matter, deficient in nitrogen and phosphorus (Babiker, 2007). The fact that sorghum is generally grown on a yearly basis on the same piece of land with hardly any measures to restore soil fertility of the already poor soils greatly compromises biological yield (Vidhaya *et al*., 2004).Thus; repeated cultivation and harvest constitute depletion of nutrients and organic matter that are already marginal. Sorghum is constantly challenged by many above- and below-ground pests and pathogens because of the range of environments in which it is cultivated. It also faces steep competition for nutrients, moisture, and light from a wide range of annual and perennial weed species such as *Andropogon* spp., *Brachiaria* spp., *Cynodondactylon, Cyperus rotundus*, *Digitaria* spp., *Echinochloacolona*, *Eleusine indica, Euphorbia*spp.and *Portulaca oleracea* (Holm *et al*., 1977., Ogborn, 1980). The effects of diseases and weeds and the inherent low fertility of soils on which sorghum has to strive are aggravated by the vulnerability of sorghum to damage by the obligate root hemi-parasitic weeds *Striga* spp. A conservative estimate, made 3 decades ago, indicated that over 20% of the area is infested by the parasite (Babiker, 2007).

2.2.2. Allelopathyin Sorghum:-

The allelochemical sorgoleone, produced and released from the root hairs of sorghum, is responsible for the observed phytotoxic effects of sorghum (Czarnota *et al*., 2001; Dayan *et al*., 2009). Sorgoleone has the potential to become a new natural herbicide (Dayan *et al*., 2009). Further, the weed suppressive activity of sorghum can be an integral part of an integrated weed management strategy.Sorgoleone, excreated by root hairs of sorghum species (Czarnota *et al*., 2001; Dayan *et al*., 2009), along with a variety of structural analogues (Kagan *et al*., 2003), is responsible for the weed inhibiting properties

2.3. Parasitic plants:-

Parasitic plants are a major threat to day's agriculture and provide an intriguing case of pathogenesis between species of relatively close evolutionary ancesvtry (Spallek *et al*., 2013). Parasitic plants vary in their plasticity and ability to occupy diverse ecological zones (Sun, 2008). Similar diversity occurs also in the degree of host specificity. Over 4100 species, in approximately 19 families of flowering plants, are able to directly invade and parasitize others plants (Nickernt and Musselman, 2004; Press and Phoenx, 2005). However, only very few of these weedsparasitize cultivated plants. Nevertheless, these weedy parasites pose a tremendous threat to the world economy, mainly because they are at present almost uncontrollable (Parker and Riches, 1993; Gerssel *et al*., 2004). These parasites are, at least in part, dependent on their hosts for the supply of carbon, nutrients, and water (Rogers and Nelson, 1962; Parker and Riches, 1993).

The families which include species of importance as parasitic weeds are seven, the most important of which are i) Orobanchaceae which include *Striga*, *Alectra*, *Orobanche*, *Phelipanche* and *Aeginetia*, ii) Convolvoulaceae comprising of *Cuscuta* spp, iii) Loranthaceae comprising

*Loranthus*and *Tapinanthus* spp., iv) Viscaceae including *Viscum album* and v) Lauraceae comprising *Cassytha ciliolata* (Sauerborn *et al*., 2007). The widest spread and important parasitic angiosperms belong to the genera *Orobanche*, *Striga* and *Cuscuta*. The most important economical hosts belong to the Poaceae, Asteraceae, Solanaceae, Cucurbitaceae, and Fabaceae (Cubero *et al*., 1994).

Parasitic plants exist in various life forms, including trees (Santalaceae), Shrubs (Mistletoes) vines (Convolvoulaceae) and herbs (Orobanchaceae) (Parker and Riches,1993; Sun, 2008). Parasitic angiosperm may be classified in a variety of ways. However, the two most obvious classifications depend on the basis of their site of attachment to the host, and the presence or absence of chlorophyll (Miller, 1994). Accordingly, parasitic plants could be classified as root or shoot parasites depending on the position of the haustorium (organ of attachment) either above or below ground (Parker and Riches, 1993; Sun, 2008). They could also be classified as holoparasites when achlorophyllus, hemiparasite when chlorophyllous and depends, at least inpart, on their hosts and facultative when they can survive without a host, but grow and yield better in prescence of a host. Parasitism could also be obligatory when the parasite needs a host at least in one or more of the stages of its life cycle.

The genus *Striga* consists of obligate hemi parasitic weeds, some of which are serious agricultural pests (Parker, 2009).They are a major biotic constraint and a serious threat to subsistence cereal crops (Pearl millet, finger millet, sorghum, maize and upland rice) grown in sub-Saharan Africa and India (Rispail *et al*., 2007; Teka, 2014). *S.hermonthica* (Del.) Benth. and *S. asiatica* (L.) Kuntze are the most economically important parasitic weeds (Ejeta, 2007; Atera *et al*., 2011) that infect sorghum, maize upland rice and pearl millet *Striga* spp. are now often identified as the greatest biological constraint to food production, and have been estimated

to infest 64% of the total cereal production area in West Africa (Gressel *et al*., 2004; Ejeta, 2007; Parker, 2012), and are continuing to expand. Infection of crops can result in grain yield losses of 20-80% in Africa but up to 100% in worst situations, and as consequence, have a significant negative impact on food security in these regions (Gurney *et al*., 2002)

2.3.1. *Striga*:-

Striga is the Latin word for 'witch'. Witchweed, Mukarram (Shuwa Arab), Maakasha or wutawuta (Hausa) and other common names for *Striga* often refer to the word 'witch', fire or killer presumably because plants diseased by *Striga* display stunted growth and an overall drought-like phenotype long before *Striga* plants appear. *Striga* species are annual plants and most of their life cycle occurs underground (Spallek *et al*., 2013).The genus *Striga,* recently placed in the Orobanchaceae (Olmstead *et al*., 2001), includes the economically important witchweeds. Mohamed *et al*. (2001) described 28 species and six subspecies from Africa. Of these 22 species are endemic, but only 11 are renowned to attack crops of economic importance. However, of all species *Striga asiatica* (L.) Kuntze and *Striga hermonthica* Del.) Benth. on cereals and *S. gesnerioides* on legumes and various other crops are the most important. These species are pre-dominant in sub-Saharan Africa (SSA) causing severe constraints to crop production. *Striga* species survive by diverting essential nutrients, from their hosts. Underground the weed siphons water and nutrients for its growth, while above the ground, the crop withers and grain yield is reduced (Khan *et al*., 2007).Crop yield loss due to *Striga* attacks can vary depending on *Striga* density, soil fertility, rainfall distribution, the host species and the variety grown.Symptoms displayed by infected hosts include severe stunting of the main stem with internodes failing to elongate properly, yellowing of leaves, wilting, chlorosis, and an increase of root: shoot ratio, reduced photosynthetic rate, increased photorespiration and low grain yield

(Doggett, 1965; Parker and Riches, 1993; Gurney *et al*., 2000). *S. hermonthica* can affect its host in different ways. The only part of the reduction in the growth of the host results from competition for carbon assimilates, water, mineral nutrients and amino acids (Graves *et al*., 1990). However, *Striga* does not only act as an additional sink but the parasite also has a strong 'toxic' or 'pathological' effect on the host (Press and Gurney, 2000).

2.3.2. Morphology:-

The genus *Striga*, erected by an Italian botanist, Loureiro, in 1790, is characterized by opposite leaves, irregular flowers with a corolla divided into the tube and spreading lobes, herbaceous habitat, and parasitism (Musselman, 1987). The flowers are pink, red, white, purple or yellow. One *Striga* plant produces a large number of tiny seeds (up to 100,000) measuring 0.3 x 0.15mm in size with a longevity of up to 20 years (Ejeta *et al*., 1993). The seeds are spread by wind, shared use of contaminated farm implements and contamination of grain stock (Weber *et al*, 1995). Normally seeds mature and are shed onto soil towards the end of the rainy season. Freshly harvested seeds remain dormant for several months depending on the species, strain and environmental conditions under which the seeds were produced (Ejeta *et al*., 1993). This period referred to as after–ripening.

2.3.3. Geographical Distribution of *Striga* species:-

Striga spp. are generally native to semi-arid, tropical areas of Africa, but have been recorded in more than 40 countries (Ejeta, 2007; Vasey *et al*., 2005). *Striga* possibly originates from a region between the Semien Mountains of Ethiopia and the Nubian Hills of Sudan (Atera and Itoh, 2011).This region is the birthplace of domesticated sorghum (Ejeta, 2007).

Approximately 30 *Striga* species have been described and most parasitize grass species (Poaceae). *Striga gesnerioides* (Wild) Vatke is the only *Striga*

species that is virulent on dicotledonous plants (Mohamed and Musselman, 2008). Among the 22 species of *Striga* prevalent in Africa, *Striga hermonthica* is the most socio-economically important weed (Gressel *et al*., 2004; Gethi *et al*., 2005).

2.3.4. Host range:-

The genus *Striga* (Orobanchaceae,) includes over 40 species, of which 11 are obligate root parasitic plants of agricultural crops (Ejeta, 2007). *S. asiatica*, S. *hermonthica* and *S. gesnerioides* are the most economically important species of *Striga* and causes considerable losses to many major African crops. *Striga asiatica* and *S. hermonthica* attack many cereal crops such as sorghum, maize, millet and upland rice.*S. gesnerioides* parasitizes only dicotyledons plants and causes considerable yield losses to cowpea [*Vigna unguiculata* (L.)Walp.], an important crop legume in sub-Saharan Africa.It also parasitizes other wild legume genera including *Alysicarpus, Indigofera, Tephrosia* and non-legumes viz*Ipomoea, Jaquemontia, Euphorbia* and *Nicotiana* spp. (Mohamed *et al*., 2001).

2.3.5. Economic Impact:-

S.hermonthica is a wide spread and severe problem in Burkina Faso, Gambia, Mali and Senegal. The occurrence of the parasite was somewhat localized in Niger and Chad. Reports from Cameroon, Nigeria, Sudan and Ethiopia revealed that crop yield is often reduced, due to *Striga* damage, by more than 60 % (Dogett, 1965).There were wide variations in levels of infestation between countries, districts, fields and within afield. The infested area was estimated in 2007 to be, 3, 1.4 and 3.5 million hectares in East Africa, central Africa and Southern Africa, respectively (Ejeta *et al*., 2007). *Striga* is claimed to affect the livelihood of some 300 million people across Africa causing moderate to severe crop damage in 43 countries (Berner *et al*., 1996; Aliyu *et al*., 2004; Ejeta *et al*., 2007).In total, 25African countries reported *Striga* infestations in 2005 (De Groote *et al*.,

2008). In monetary terms the economic damage instigated by the parasite is equivalent to approximately 1 billion US$ per year (Labrada, 2008; Waruru, 2013). Farmers may eventually be forced to abandon highly infested fields (Atera and Itoh, 2011).

2.3.6. Life cycle of *Striga*:-

Striga spp. are obligate hemi-parasitic plants that attach to the root of their host to obtain water, nutrients, and carbohydrate (Parker and Riches, 1993). Most *Striga* species have a very complex life cycle (Plate 2.1). The seed of *S .hermonthica* is small dust-like and measures 0.2 to 0.4 mm (Parker and Riches, 1993). Energy reserves in seeds are limited and sufficient only for a short period of autonomous growth (Doggett, 1965). *Striga* is completely dependent on the host for its survival, and its life cycle is closely linked with that of the host plant (Haussmann *et al*., 2000). The life cycle of *Striga* is divided in to an independent non-parasitic or vegetative phase and a parasitic one (Mohamed *et al*., 1998). The non-parasitic phase, includes germination and radicle extension.The parasitic phase starts on the initiation of a haustorium followed by attachment, penetration, and the establishment of connection with the host xylem and further development of a mature plant that flowers and sets seeds (Haussmann *et al*., 2000). Striga seeds have after-ripening requirements which may extend for 2 to 6 months depending on climatic conditions, humidity and temperature (Rich and Ejeta, 2007). During after-ripening, certain internal changes, of which little is known, take place gradually inside the seed **.**After-ripened seeds will not germinate until they have passed through a pre-treatment period in warm moist conditions for 2-14days and subsequently exposed to a germination stimulant (Parker and Reid, 1979). The duration and temperature optima for the conditioning period vary with the species. In *S. hermonthica* the optimum conditioning period is two weeks at 33 ºC (Parker and Reid, 1979). The biochemical changes that occur during conditioning are not well known. However, conditioning is presumed

to reduce germination inhibitors within the seed (Kust, 1966). A chemical stimulant is needed in order to trigger the germination of root parasites (Press and Graves 1995; Yasuda *et al*., 2003). However, some preparatory metabolic processes take place before the seed can react to the respective germination stimulant. The first natural germination stimulant Srigol was obtained from cotton (Cook *et al*., 1972). Once triggered by the stimulant and at a favorable temperature, seeds germinate in 24 hours (Ramaih. 1985). On contact with a host root, the tip of the radicle swells and produces a specialized organ the haustorium by which it attaches to the host root. *Striga* seedlings survive only if they succeed to attach to the host root within five days following germination (Ejeta *et al*., 1993). Once connected, the parasite withdraws water, mineral nutrients, carbohydrates and amino acids, consequently causing stunted shoot growth, leaf chlorosis and reduced photosynthesis in the host. After several weeks of underground development, the *Striga* shoots emerge above the soil surface and start to flower and produce an extremely high number of seeds (up to 100,000 seeds/plant) that remain viable for as long as 20 years (Kroschel and Müller-Stöver, 2004). Pre-conditioned seeds, not exposed to a stimulant, enter a period of wet-dormancy which to last on drying. This is considered a survival mechanism that helps to build a seed bank of *Striga* in tropical soils (Ejeta *et al*., 1993).

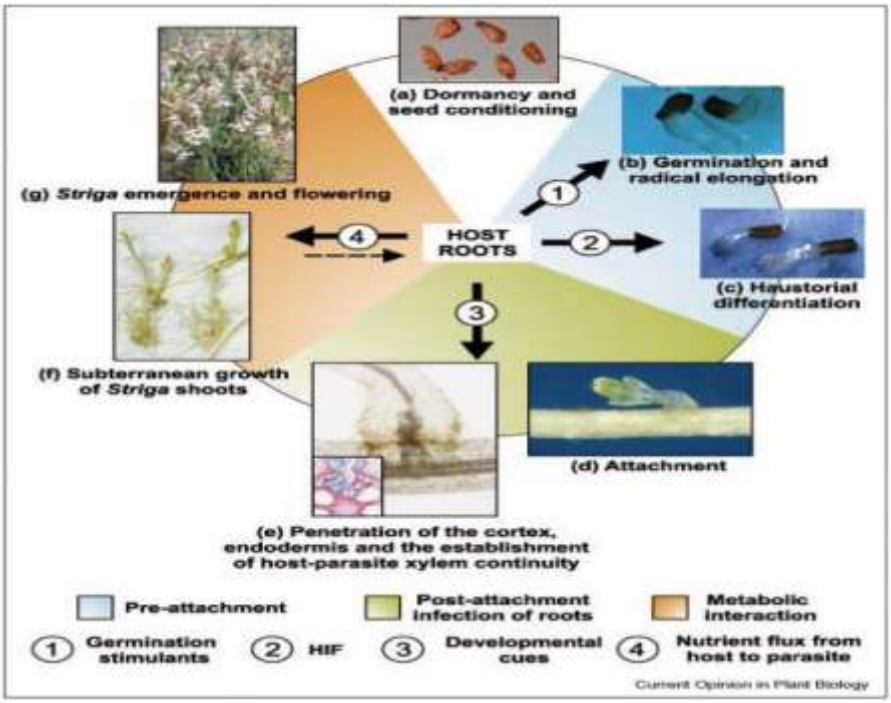

Plate 2.1.The lifecycle of *Striga* adaptedwith few modifications from Scholes and Press (2008).

2.4. Control methods:-

Several control measures,including preventive, mechanical and cultural methods have been tried for control of parasitic weeds, So far these methods, however, have only had a limited impact on the parasites and up-to-date there is no single control method that can effectively solve the problem (Joel, 2000, Ejeta, 2005).

2.4.1. Preventive methods:-

One of the most important control methods is to prevent the introduction and distribution of the parasite seeds from one field to another and from infested to uninfested areas. Short distance transport, within a field and between fields, can be achieved by wind, water, grazing animals and soil on tools or farm equipment (Sun, 2008). Long distance transport is attained mainly through national and international trade of crop seeds and other commodities. Infestation of *S. asiatica* in North and South Carolina in the

US is attributed to a contaminated wool consignments from South Africa (Babiker, 2007). For effective prevention stricts anitation measures should be adhered to. Infested areas have to be located, isolated and movement of farm equipment, farm products and associated materials have to be carefully manipulated and examined for contaminations by the parasite seeds.

2.4.2. Cultural methods:-

2.4.2.1. Hand weeding:-

Hand weeding is recommended to prevent seed set and seed dispersal. Weeding small *Striga* plants is a tedious task and may not increase the yield of already infected plants. However, it is necessary to prevent seed production and re-infestation of the soil. Due to high labour cost in repeated hand pulling of *Striga* it is recommended that hand pulling has to begin 2-3weeks after *S. hermonthica* starts flowering to prevent seeding (Parker and Riches, 1993). New shoots may sprout out after cutting or pulling from infected plants requiring a second weeding before crop maturity.

Hand pulling is the simplest and certainly the most effective method to apply to small fields with low to moderate levels of infestation. Previous work in West Africa on *Striga* on pearl millet showed that one hand weeding, just prior to harvest, significantly reduced infestation in a subsequent pearl millet crop and increased yield (Ramaiah, 1985).

2.4.2.2. Trap cropping:-

In-theory trap-crops, plants which stimulate *Striga* seeds germination without being parastized, should reduce Striga seed bank through suicidal germination and improve yield of the subsequent cereal (Ahonsi *et al.,* 2002).However, reports on success of trap crops are controversial. Several crops including some varieties of cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*), soybean (*Glycine max*) and sesame (*Sesamum*

indicum), were reported to have potential to induce suicidal germination of *S. hermonthica* and improve soil fertility (Carsky *et al*., 2000; Hess and Dodo, 2003;Schulz *et al*., 2003).In Gambia rotation of early pearl millet and groundnut, on a seasonal basis, followed by grazing and space tethering of cattle at night, in the cereal stubble during the off season, caused considerable reduction in *Striga* infestation (Carson, 1989).However, rotation with trap crops is reported to be less effective in reducing *Striga* seed bank in the dry and less humid East Africa. Work in Eastern Kenya showed that 4 years of continuous cropping with cowpea or cotton did not reduce *Striga* infestation below damaging levels (Ransom, 2000).

The germination inducing activity of root exudates of trap crops could be attributed to the ubicotous nature of Strigolactones in planta (Cardoso *et al*., 2011). Strigolactones are reported to be extremly labile in soils and could only display limited mobility particularly in heavy alkaline soils. Unstability of strigolactones in soils controls the distance where effective germination could be affected. A proposed solution for increasing efficiency and consistency of trap crops is to increase sensitivity of the parasite seeds to germination stimulants where the seeds could respond a long a stimulant concentration gradient. Germination of parasitic is reported to be govered by the ratio of GA to ABA in the seeds. Joel (1995) reported synthesis of GAs in *Orbanche crenata* seeds during conditioning. Degradation of ABA or increasing GAs in seeds through chemical manipulation should be exploited.

2.4.2.3. Catch cropping:-

Catch cropping is a method which employs planting true hosts at high densitys to reduce Striga seed bank to a non-damaging level.The catch crop is to be sacrificed by ploughing in within 6-8 weeks after emergence to prevent attached Striga plants from setting seeds. The main crop could then

be planted during the main rains (Parker and Riches, 1993). Catch cropping is a suitable method for curbing the parasite in regions endowed with long rainy seasons or in areas with dual rains or under irrigation. However, it is definitely not suitable in places where short rainy seasons are pre-dominant.

2.4.2.4. Intercropping:-

Intercropping cereals with legumes and other crops is a common practice in most areas of Africa and has been reported to influence *Striga* infestation. Intercropping is a potentially viable, low-cost technology, which would enable addressing the two important and interrelated problems of low soil fertility and *Striga* (Fasil, 2002). Growing sorghum in association with cowpea and haricot bean (*Phaseolus vulgaris*) was reported to be effective against *S. hermonthica* and to improve, significantly, yields of the subsequent cereal (Fasil, 2002). Intercropping maize with cowpea and sweet potato significantly reduced emergence of *Striga* (Oswald *et al*., 2002).In Kenya, more recently, it was discovered that inhibition of *S.hermonthica* infection was significantly greater in maize-silver leaf [*Desmodium uncinatum* (Jacq.) DC.] inter crop than that observed with other legumes, for example, sun hemp (*Crotolaria* spp.), soybean or cowpea (Khan *et al*., 2000). Consequently, the yield of maize was significantly increased by two tons/ha. *Desmodium* spp. are legumes that can easily be controlled by regular cutting in order to avoid or minimize competition with the crop if any.

According to Khan *et al*. (2007), intercropping different legumes with maize and sorghum helps reduce *Striga,* but does not eliminate the weed. This explains why, inspite of most farmers intercropping cereals with legumes as the dominant cropping system in western Kenya, *Striga* infestation is still high in most fields. A variant of an inter-cropping system dubbed “push-pull” where *Desmodium* spp. is intercropped with cereals

with an edge of fodder crops is effective in *Striga* management. There is, therefore, aneed to combine more than one control method to improve the effectiveness of existing control strategies (Ejeta and Gressel, 2007). Research results from Germany using hyacinth bean (*Lablab purpureus*) (Dawoud, 1995) and Kenya, using silver leaf, [*Desmodium uncinatum* (Jacg.) DC.] and Green leaf [D. *intorum* (Mill.) Urb] (Khan *et al.,* 2002) showed that the action of intercrops may be much more complex than originally thought and indicated exudation of allelopathic compounds which induce pre-mature haustoria, curtail radicle extension and thereby decrease attachment and parasitism without influencing germination. *Desmodium* spp., apart from successful suppression of the parasite and increasing grain yield by several folds, are repellent to the stem borers *Busseolofusca* (Noctuidae) and *Chilopartellus* (Pyralidae), excellent nitrogen fixers (100-180kg nitrogen/ha), preserve soil moisture and provide high-value fodders (Khan *et al.*,2002).

Intercropping with *Desmodium* spp. represents a platform technology around which new income generation components such as livestock keeping can be built. At present intercropping with *Desmodium* spp. to compact *Striga* and insect pests in maize is adopted by over 6 thousand farmers in Western and Eastern Uganda (Khan *et al.,* 2007).

2.4.2.5. Crop rotation:-

Rotation with non-host crops interrupts further production of *Striga* seed and leads to decline in the seed population in the soil. The practical limitation of this technique is it requires more than three years to be effective (Parker and Riches, 1993). The choice of the rotational crop should, therefore, be based first on its suitability to the local conditions and only secondarily on its potential as a trap crop (Parker and Riches, 1993). Rotating the infested maize or sorghum areas to wheat/barley, pulses, or groundnuts are viable and effective options in Ethiopia (Shank, 2002).

In Ethiopia, two years of cropping to a non-host were reported to reduce *Striga* infestation by 50% (Shank, 2002). In the Sahel, the results of a four-year experiment in bush fields indicated that one season cowpea in 1998, had a positive effect on subsequent pearl millet grain yields, soil organic carbon and nitrogen, and *Striga* infestation. The increase in yields due to the pearl millet-cowpea rotation was 37% in 1999 compared to three to five year's continuous millet cropping (Samake, 2003). However, small-holder farmers desiring to maximize the grain production potential of their land may be difficult to be persuaded to grow other crops. Practical control measures are effective when a combined program of crop rotation, weeding, sanitation and, resistant varieties is included.

2.4.2.6. Resistant and tolerant varieties:-

Host plant resistance would probably be the most feasible and potential method for parasitic weed control. Using biotechnological approaches, including biochemistry, tissue culture, plant genetics, breeding and molecular biology a significant progress has been made in developing screening methodologies and new laboratory assays, leading to the identification of better sources of parasitic weed host resistance (Ejeta *et al*., 2000; Haussmann *et al*., 2000; Omanya, 2001). It is potentially an acceptable *Striga* control option to resource-poor farmers (Gurney *et al*., 2003; Rich *et al*., 2004). However, reliance on host resistance alone is not ideal because so far complete resistance against *Striga* cannot be attained through breeding (Gurney *et al*., 2002), and usually, the newly developed varieties may not fulfill farmers preference traits (Adugna, 2007). Reports of genetic resistance to *Striga* have been documented in rice (Bennetzen *et al*., 2000; Gurney *et al*., 2006), sorghum (Haussmann *et al*., 2004; Mohamed *et al*., 2003; Rich *et al*., 2004), cowpea (Riopel and Timko, 1995) and maize (Adetimirin *et al*., 2000; Menkir, 2006). Identifying source germplasm with different resistance mechanisms facilitates

combining several resistance genes to obtain more durable and stable polygenic resistance to *Striga* in cereals (Ejeta *et al*., 2000; Menkir, 2006). Various molecular markers are also available for genetic analysis such as restriction fragment length polymorphisms (RFLPs) (Perumal *et al*., 2007), random amplification of polymorphic DNAs (RAPD) (Agrama and Tuinstra, 2003), amplified fragment length polymorphisms (AFLP) (Perumal *et al*., 2007), microsatellites or simple sequence repeats (SSRs) (Ganapathy *et al*., 2012) and single nucleotide polymorphisms (SNPs) (Arai-kichise *et al.*, 2011). Various studies have reported combined use of phenotypic and molecular markers in genetic analyses of cereals such as rye grass (Jianyang, 2005), rice (Ogunbayo *et al*., 2005), maize (Beyene *et al*., 2005; Wende *et al*., 2012), and sorghum (Bucheyeki *et al*., 2009; Agrama and Tuinstra, 2003).

Resistant varieties have been highlighted as the most effective and environmentally sound method for the control of *Striga*. This has been demonstrated in multi-location field tests conducted in Ethiopia and Tanzania (Mbuwaga *et al*., 2007; Tesso *et al*., 2007). The International Institute for Tropical Agriculture (IITA 2002) has released *Striga* resistant, drought-tolerant, and low soil nitrogen-tolerant extra-early maturing white maize varieties in Nigeria. There are also *Striga* resistant/tolerant maize hybrids and varieties released in West Africa.

More than 80 resistant sorghum lines have been selected by the International Center for Dryland Research (ICRSAT) in India. Recently, of these, some high yielding *Striga* resistant sorghum and millets varieties have been made by the Ethiopia Institute of Agriculture Research at Nazreth, and introduced and registered in the country (Adugna, 2007; Ejeta, 2007). These varieties, when deployed along with moisture conservation practices and soil amendment inputs, can dramatically reduce *Striga* infestation and increased sorghum yield by up to 400%. However,

adoption of these varieties has been slow primarily due to the introduced germplasm does not fulfill farmers preferred traits (Adugna, 2007), and lack of effective seed production and delivery mechanism. Purdue University in the USA also identified and recommended two sorghum varieties; P9401 and P9403, for full commercial production. These varieties combine excellent grain quality and drought tolerance. They have been highly preferred by Ethiopian farmers, they were named Gubiye (P9401) and "Abshir (P9403) that are resistant or tolerant to Striga .Hiriray, Higretay and Korokora are Ethiopian maize varieties that are resistant due to their early maturing characters which is an escape mechanism from *Striga* infection (Kidane *et al.*, 2004). Promising results were also obtained in sorghum when both traits, *Striga* and drought resistance, were combined by classical breeding. Basically, the resistant varieties were low yielding and not desirable in other agronomic characteristics. However, integrating genetic resistance with other control measures is the smartest option possible both for the effectiveness of control as well as for increasing durability of resistance genes (Ejeta, 2007).

2.4.2.7. Soil fertility:-

Nitrogen and phosphorus deficiency, as well as water stress, accentuate the severity of *Striga* damage (Yoneyama, 2012). *Striga* is particularly a pest of low fertility soils and usually the infection decreases if mineral nutrients, especially nitrogen and phosphorus, are applied in sufficient quantities (Adagba *et al*., 2002).

Fertilizer had a significant effect on height, vigor score, reaction score of sorghum as well as shoot count, days to emergence, dry matter production and dry weight of Striga. Nitrogen (N) at high rate increases performance of cereal crops under *Striga* infestation. This is due to the fact that nitrogen reduced the severity of *Striga* attack while simultaneously increasingly the host performance (Lagoke and Isah, 2010).

Results of an experiment, designed to develop an integrated nutrient management strategy, confirmed that the combined use of 41 kg N/ha and 30 t/ha of manure could lead to significant reduction in Striga infection and a considerable increase in sorghum yield (Esilaba *et al*., 2000). Esilaba *et al*. (2000) and Gacheru and Rao (2001) also found that increasing soil fertility not only stimulates the growth of the host but also adversely affects the longevity of Striga seeds in soil, their germination, and seedlings attachment. High dosages of nitrogen fertilizer are generally beneficial in delaying *Striga* emergence and obtaining vigorus crop growth (Dugje *et al*., 2008). Also, other advantageous effects of fertilizers include increasing soil nitrogen and other nutrients, replenishing the organic matter and increasing soil moisture holding capacity (Ikie *et al*., 2006).

2.4.3. Biological methods:-

The objective of biological control is not the eradication of weeds but the reduction and establishment of a population below the economic threshold level (Rajni and Mukerji, 2000). Means of biological control of weeds comprise herbivorous insects, microorganisms (especially fungi), and smotheringcrops (Sauerborn and Kroschel, 1996). The method, involves importation, colonization, and the establishment of exotic natural enemies, which include predators and parasitoids. Efforts to manage weeds using biological control have been gaining momentum throughout the world, especially in the recent past (Delfosse, 2004). Biological control is considered as a potentially cost-effective, safe and environmentally beneficial alternative mean of reducing weed populations in crops, forests or rangelands (Charudattan, 2001). Disadvantages of weed biological control include the long period (5 to10 years) required for research and a high initial investment of capital and human resources (Culliney, 2005). Biological control is unattractive as a private entrepreneurial effort (Hill and Greathead, 2000).

2.4.3.1. Biological control using insects:-

The insects that attack *Striga* can be classified according to their damage as defoliators such as *Junonia* spp., gall forming as *Smicronyx* spp. (Coleoptera: Curculionidae) in India and Africa; shoot borers as *Apanteles* spp., miners as *Ophiomyiastrigalis*, Spencer (Diptera: Agromyzidae) in East Africa; inflorescence feeders as *Stenoptilodes taprobanes* and fruit feeders as *Eulocastra* spp. (Lepidoptera: Noctuiidae) in India ;(Kroschel *et al*., 1999).

In the 1990s, studies in Burkina Faso and Northern Ghana have been carried-out by Jost *et al*. (1996) and Traoré *et al*. (1996) to investigate the potential of the weevils *Smicronyx guineanus* and *S. umbrinus* and the butterfly *Junonia orithya* as biocontrol agents for *Striga*. As a result of *Smicronyx* infection, the *Striga* seed production was reduced by 17.4% on the average (Kroschel *et al*., 1999). Kroschel *et al*. (1999) concluded that the use of herbivorous insects could play a role in an integrated control package, lowering the *Striga* population by reducing its reproduction capabilities and spread. However, the augmentation of native insect populations through inundative releases is not applicable in the third world, mainly due to the infeasibility of mass rearing.

2.4.3.2. Biological control using pathogens:-

Most organisms have natural enemies that balance their populations, avoiding excessive abundance (Templeton, 1982). Biological control of *S. hermonthica* using *Fusarium oxysporum* is considered as one of the novel management strategies (Sauerborn *et al*., 2007). Fungi are preferred to other microorganisms as bio-herbicides because they usually host specific, highly aggressive, and easy to mass produce and are genetically diverse (Ciotola *et al*., 2000). Field and laboratory tests showed that *F. oxysporum* is highly effective in hindering germination, growth, and development of

Striga and thus may lead to a reduction of *Striga* seed bank in the soil (Ciotola *et al*., 2003).

Approximately 16 fungal genera were found on *Striga* spp. (Greathead, 1984; Abbasher and Sauerborn, 1992; Abbasher *et al*., 1998).Results of surveys for fungal pathogens of *Striga* and *Orobanche* showed that *Fusarium* species were the most prominent associates of diseased broomrapes and witchweed. Of these, *F. oxysporum* was the most predominant species (Sauerborn *et al*., 2007)

Extensive surveys in Burkina Faso, Mali and Niger also demonstrated the occurrence of highly pathogenic and *Striga* specific isolates of *F. oxysporum* (Ciotola *et al*., 2000). Among the isolates, *F. oxysproum* M12-4A provided more than 90% control of *Striga*, and a three-fold increase in sorghum biomass (Ciotola *et al*., 1996). The use of a mycoherbicide, that is *F. oxysporum*coated seeds and host plant resistance reportedly reduced *Striga* emergence by 95% and increased sorghum yield by 50% (Franke *et al*., 2006).

Recent findings indicated the effectiveness of the integrated use of *F. oxysporum* and *Striga* resistant sorghum genotypes to control *Striga* in Ethiopia (Rebeka *et al*., 2013). To realize the full potential of this approach it is important to recombine traits of *Fusarium* and *Striga* resistant sorghum lines. This would allow continued selection of targeted progenies with combined resistance and *Fusarium* compatibility and for subsequent seed treatment of suitable hybrid(s) for direct use. Thus effective *Striga* control would be possible through the synergistic effect of biocontrol and host resistance.

Recently, the combined application of two or more control measures has been promoted for effective *Striga* management.The use of abio-control agent such as virulent isolate of *F. oxysporum* f.sp. Striga as a component of integrated *Striga* management was identified to have several advantages

(Ciotola *et al*., 2000; Fen *et al*., 2007) and Marley *et al*. (2004) also found that the application of integrated *Striga* management package combining a mycoherbicide based on *F. oxysporum* isolate and host plant resistance has been demonstrated in farmer's fields as effective *Striga* control approach. Several researchers reported combined use of resistant varieties with *Fusarium oxysporum* applied as pest's granules or as a seed coating are effective against *Striga* (Marley *et al*., 2004; Julien *et al*., 2009).Various *Fusarium* spp. and vesicular-arbuscular mycorrhizal (VAM) fungi have been found which can reduce *Striga* infestations significantly on sorghum and maize when used together with resistant and/or tolerant host (Ciotola *et al*., 2000; Lendzemo *et al*., 2005; Franke *et al*., 2006).

2.4.4. Chemicals methods:-

This method employs a diversity of chemicals including germination stimulants, herbicides, and fumigants.

2.4.4. 1.Germination stimulants:-

Induction of *Striga* seed germination in absence of host plants, suicidal germination, has been the subject of numerous researches since the turn of the last century (Parker and Riches, 1993). The research was focused on identification of the natural germination stimulant(s) occurring in the root exudates of plants known to stimulate *Striga* germination. Srigol, a very potent *Striga* germination stimulant, was isolated from cotton (*Gossypium hirsutum* L.) (Cook *et al.*,1972). Ethylene is regarded as a multifunctional phytohormone that regulates both growth, and senescence. It promotes or inhibits growth and senescence processes depending on its concentration, timing of application, and the plant species. The application of ethephon, an ethylene releasing compound enhanced ethylene evolution and increased leaf area of mustard at a lower concentration, while inhibited at higher concentration (Khan *et al.*, 2008).

Certain chemicals, synthetic and natural such as ethylene, ethephon, Srigol and Srigolanalogues induce germination of *Striga* seeds in the absence of a suitable host and therefore deplete seed reserves in the soil (Esilaba and Ransom, 1997). In plant species, there is evidence that the production of Strigolactone by the host plant could be reduced if sufficient minerals are available (Lopez-Raez *et al*. 2008).Strigolactones (SLs), the most potent germination stimulants of weeds, are ubiquitous in plant and through cross-talk with other hormones modulate plant growth, particularly shoots and roots architecture and in synchrony with soil fertility, particularly phosphorus and nitrogen deficiencies, they affect the composition of rhizospheric microbiome (Yoneyama, 2020). Although SLs are synthesized in the roots they are translocated to the shoots and are widely distributed within plant parts (Yoneyama *et al*. 2007b; Lopez-Raez and Bouwmeester 2008; Foo *et al*. 2013).

Typical SLs (canonical) contain a fused 6-5-5 membered ring as the ABC-ring system. The ABC-ring, the core, is connected to a methyl-butenolide moiety (D ring) *via* an enol-ether bridge. However, recently another group of SLs "non-canonical" which lack the ABC ring system, the core in their canonical congeners, but retain the enol-ether–D ring moiety which is claimed to be essential for germination inducing activity have been identified (Burn *et al,* 2018; Yoneyama, 2020). Canonical SLs are further divided, based on orientation of the C ring, into two group's strigol and orobanchol type with a *β*-oriented and an *α*-oriented C ring, respectively. In general SLs are chemically unstable and the hydroxylated *viz* orobanchol and non-canonical SLs are by far less stable than their canonical congeners in soil and aqueous solutions (Yoneyama, 2020).

Several Srigolanalogues were synthesized and proved to be as effective as Srigol in laboratory tests (Johnson *et al.*, 1981; Ibrahim *et al*., 1985). However, the compounds displayed a complete loss of activity within one

day when incubated in heavy alkaline soils (Babiker *et al.*, 1988). The research focused on sorghum (true host) root exudates led to the discovery of two compounds with high germination inducting activity, dihydrosorgoleone and a sorgolactone (Chag *et al*., 1986; Hauck *et al*., 1992).The compounds dihydrosorgoleone, a reduced benzoquinone, and sorgolactone, a Srigolanalogue, proved to be highly unstable (Joel *et al*., 1995). The extreme instability of these compounds poses a serious limitation on their use under practical field conditions. However, it may be possible to increase the persistence of these compounds if a controlled-release technique is adopted. Utilization of such techniques has been reported to slow down the degradation of many pesticides (Shasha *et al*., 1981). Other limitations to commercial utilization of these compounds include cost and market availability.

2.4.4.2. Herbicides:-

2.4.4.2.1. Pre-emergence herbicides:-

The technology currently being deployed as a complement to *Striga* resistance in maize involves the use of herbicide as a seed coating. This has led to the emergence of a new technology known as imazapyr- resistant maize (IRM) which has proven to be efficient for *Striga* control (Kanampiu *et al*., 2004; De Groote *et al*., 2006). The International Maize and Wheat Improvement Center (CIMMYT), Badische Anlin and Soda Fabrik (BASF), African Agricultural Technology Foundation (AATF) and other stakeholders have made efforts in bringing imazapyr-resistant maize (IRM) technology to farmers as assistance for *Striga* control. The result of experiments also proved that herbicide seed treatment using imazapyr appears to be a promising approach for the control of *Striga* in maize or sorghum (Dembele *et al*., 2005). Ndung'u (2009) has also reported coating sorghum seed with herbicide reduced *Striga* infestation, *Striga* flowering,

and *Striga* seed set, and it is considered as the most effective approach as it does not affect sorghum biomass.

Research on-farm trials in Kenya and Tanzania indicate that seed dressing with Imazapyr and Pyrithiobac offers good *Striga* control and increased maize yields (Kanampiu *et al*., 2004).

2.4.4.2.2. Post- emergence herbicides:-

Herbicides tested for the selective control of *Striga* mostly acts through the foliage, although some have soil residual effects. Among the herbicides tested, 2, 4-D has been the most selective and is the cheapest. 2-methyl-4-chlorophenoxyacetic acid (MCPA), a compound closely related to 2, 4-D, has also been effective especially when mixed with bromoxynil (Ejeta *et al*., 1996). 2, 4-D (1 L product/ha), glufosinate (2 L product/ha) and oxyfluorfen (1 L product/ha) applied as post-emergence treatments for control of Striga were reported to be effective in preventing the top growth of *Striga*. Babiker *et al*. (1996) reported that a combination of urea and dicamba effectively controlled *Striga* (62-92%) on sorghum, while chlorsulfuron in combination with dicamba controlled *Striga* as much as 77-100% on sorghum. However, results of the experiments showed that post- emergence herbicides do not prevent crop yield loss because they cause their impact after *Striga* has already attached and damaged the host.

Research efforts on the identification of systemic herbicides, which could ideally translocate through the host crop to prevent initial stages of parasite development, were not successful. Research efforts should, therefore, be directed towards identifying herbicides that persist in the soil, allowing the germination of *Striga* seeds but killing the seedlings before attachment to the host. Herbicides must also be compatible with the mixed cropping systems practiced by farmers and be profitable to use with low initial capital outlay.

2.4.4.3. Fumigants:-

Soil fumigation is one of the methods of control which was used in the USA for the eradication of the parasite. Methyl bromide, a liquid under pressure, was injected into the soil at a rate of 400 kg/ha^{-1}under a plastic cover to retain the gas for 24 hours.The soil has then to be ventilated for 7days before planting a crop (Eplee, 1992; Parker and Riches, 1993).

The product can effectively rid a site of an infestation through a single treatment (Eplee, 1992). However, because of high cost, labour and equipment requirements as well as health risk, the product is not considered suitable for commercial application even in the USA (Eplee, 1992). Another fumigant, dazomet, was extensively used in the USA in the witchweed eradication programme. However, the high rate 9320kg/ha and high cost precluded utilization in Africa (Eplee, 1992; Parker and Riches, 1993).

2.4.5. Integrated management:-

The level of *Striga* infestation and damage is increasing; farmers rarely adopt *Striga* control methods either due to limitations associated with the technology itself, access and costs of the technology or due to lack of information about available technology options (Oswald, 2005; Hearne, 2009).

Currently, available *Striga* control practices are only partly effective. No single management option has been found effective across location and time. The observed variability in performance may be attributed to a multitude of interacting variables including the parasite, the host, and the environment. The severity of *Striga* attack is modulated by the size of the seed bank, the existence of strains, variants, and races with different virulence, the reaction of the host cultivar and environment. The need for a strategy based on an integrated approach which focuses on crop

management practices that have been developed and tested at the field level is imperative.

Today several control options have been recommended to reduce *Striga* damage such as resistant cultivars, crop rotation, intercropping with pulse crops, late planting, deep planting, trap crops, organic and inorganic fertilizers, herbicides, and biological control (Hearne, 2009). Furthermore, available options when applied individually are not effective and sometimes affected by environmental conditions Integration of weeding with high rates of urea appropriate sowing date, and effective control of weeds which may serve as alternative hosts, will further enhance the long-term control of *Striga* (Fasil, 2002). The combined use of row planting, fertilizers, and hand pulling (during flowering) registered 48% higher grain yield and over 50% reduction in *Striga* shoot counts compared to the farmer's practice at Adibakel, in Tigray, Ethiopia (East Africa). However, from this result of research experiment showed that the best solution for the control of *Striga* is an integrated approach that includes a combination of methods that are affordable and acceptable to farmers.

According to the research findings, the integration of multiple control options is suggested as a better approach to combat *Striga* problem (Kuchinda *et al*., 2003; Schulz *et al*., 2003, Aliyu *et al*., 2004; Temam, 2006; Tesso *et al*., 2007). Schulz *et al*. (2003) and Hearne (2009) also proved that the best options for successful *Striga* control lie in an integrated approach.

CHAPTER THREE

MATERIAL AND METHODS

3.1. General

A series of laboratory and greenhouse experiments were undertaken at the College of Agricultural Studies, Sudan University of Science and Technology (SUST) at Shambat,(Lat22-27°N,Long8-20°E),to study i) The effects of sorghum root exudates and residues on *Striga* germination, ii)The possibility of using sorghum residues for *Striga* management, iii)Distribution and effects of soil placement on persistence of stimulants from sorghum residues iv)Effects of drying method on activity of sorghum residues on *Striga*.

3.2. Laboratory experiments:-

3.2.1. Materials:-

3.2.1.1. Plant materials:-

Three genotypes, WadAhmed *Striga* tolerant improved variety, Tetron, *Striga* resistant local landrace (Nasreldin *et al*., 2016) and Hakika *Striga* resistant exotic genotype were obtained from the Agricultural Research Corporation (ARC), Wad-Madni, Sudan.

3.2.1.2. Seed cultivars surface sterilization:-

The seeds of sorghum cultivars were dipped for surface-sterilized in1% sodium hypochlorite (NaOCl) solution for 5 min. After thorough rinsing with sterilized distilled water for several times, the seeds were air dried and kept in sterile bottles till used.

3.2.1.3. *Striga* seeds cleaning and surface sterilization (disinfection) :-

Striga seeds (0.5-1g) were poured into a measuring cylinder (1000 ml), filled with tap water to which Tween 20 (0.5-1ml) was added. The

measuring cylinder was occasionally swirled, the seeds were allowed to settle and water containing debris and light seeds were decanted. The heavy seeds, separated from sand by repeated flotation and decantation, were subsequently transferred to a fine sieve (70µm) and washed with tap water several times to remove traces of the detergent. The seeds plotted dry on Whatman No1 filter papers, were air dried and stored at ambient temperature till used.

Seeds were surfaced sterilized (disinfected) by immersion, for 3min, in sodium hypochlorite (NaOCl) solution (1%), obtained by appropriate dilution of commercial sodium hypochlorite solution (Bleach containing 5%NaOCl). The sodium hypochlorite was drained off. The seeds were washed, under suction, with sterilized distilled water several times, until the yellow color disappeared. The seeds blotted dry on Whatman No1 filter papers, were air-dried under a laminar flow cabinet and subsequently stored till used.

3.2.1.4. *Striga* seeds pre-conditioning:-

Glass fiber filter papers (GFFP) discs (8mm diameter) were cut, wetted thoroughly with water and placed in an oven set at 104 ºC for one hour to be sterilized before use. For pre-conditioning, the sterilized discs, placed in 9 cm Petri dishes lined with a single sheet of glass fiber filter paper, were moistened with 5 ml of distilled water. Subsequently, about 25-50, surface sterilized *Striga* seeds were sprinkled on each of the glass fiber discs. The Petri dishes sealed with Parafilm to avoid moisture loss were wrapped with aluminum foil, and incubated in the dark at 30 ºC, for 14 days.

For germination glass fiber filter papers discs containing conditioned *S.hermonthica* seeds, dapped on a filter paper to remove excess water, were transferred to sterile Petri dishes. Each disc was treated with a 25µl aliquot, of the respective test solution. A piece of filter paper, moistened with sterilized distilled water, was placed in the center of each Petri dish to

maintain moist conditions during the test period. The seeds re-incubated in the dark at 30 ºC, were examined for germination 24 h later using a binocular stereomicroscope. Treatments were arranged in a Complete Randomized Design (CRD) with 4 replicates. All experiments were repeated at least Two times.

3.2.1.5. Chemicals:-

3.2.1.5.1. Strigolanalogue (GR24) stock solution:-

A stock solution of the synthetic germination stimulants GR24 (Fig3.1) was prepared by dissolving 1mg in 1ml of acetone and completion to volume (100 ml) with sterilized distilled water to obtain the desired concentration (10 ppm).

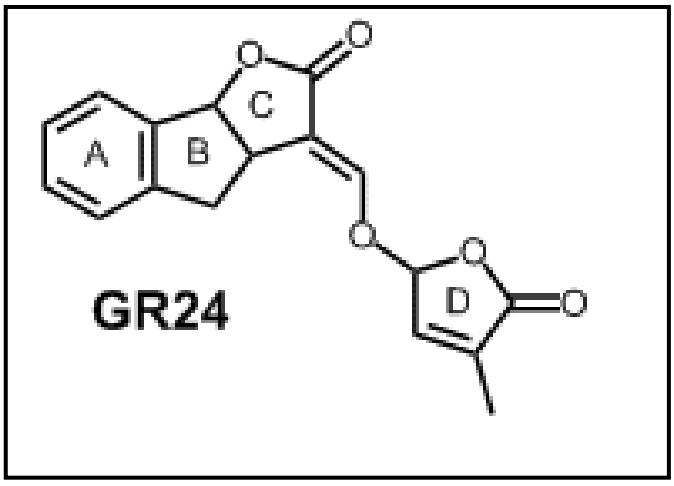

Fig.3.1. Chemical structure of the synthetic *Striga* germination stimulant GR24

3.2.1.6. Preparation of Agar medium:-

Low nutrient agar medium (gelling temperature 30-31ºC, NacalaiTesque, Kyoto, Japan) was prepared by adding 3 g to one liter of distilled water and subsequent autoclaving at 15 bars and 121 ºC for 15 minutes. The temperature of the autoclaved agar was was adjusted 40 °C using a temperature controlled water bath.

3.2.2. Methods:-

3.2.2.1. Germination, attachment and survival of *S .hermonthica* as influenced by sorghum genotype: -

In-situ germination and subsequent development of *S. hermonthica* on three sorghum genotypes, WadAhmed (*Striga* tolerant improved variety), Tetron (*Striga* resistant local landrace), and Hakika (*Striga* resistant exotic genotype) differing in their reaction and/or origin to the parasite were investigated. An in vitro system (the Rhizotron technique) for sorghum and *Striga* co-culture, adapted from Vasey *et al*., (2005) for wheat (*Triticum asetivum*) and *S. hermonthica* was used. Briefly surface disinfected sorghum seeds were germinated on filter paper for 3 days at 30°C.The seedlings were subsequently transferred to glass test-tubes filled with 40% long Ashton solution. The tubes were wrapped with aluminum foil to exclude roots from light. Plants were allowed to grow for 10 days in a controlled environment with a 12 h photoperiod, a temperature of 30°C and subsequently transferred, each, to a rhizotron. The rhizotron comprised of a 150 mm diameter plastic Petri- dish with a sheet of rock wool at the bottom overlaid by a glass fiber filter paper. The rhizotron had a hole at the top to allow for shoot growth (Plate 3.1). S. *hermonthica* seeds surface sterilized and conditioned in distilled water, as described by Babiker, *et al*., (2000), were sprinkled in close proximity of the roots. The rhizotron, placed in a black jacket, each, were incubated in a controlled environment with a 12 h photoperiod and a temperature of 30 °C prior to examination for *Striga* germination, attachment and seedling development at 7, 14- and 21-days post inoculation. Treatments were arranged in a complete randomized design with 4 replicates. Germination counts and seedlings development were based on 200 seeds, randomly selected, in each rhizotron. Attachments were classified according to their stage of development from

stage 1 (least advanced) to stage 3 (most advanced): stage 1 = parasite plumule emerged from seed coat, cotyledons visible, stage 2 shoot formed two leaf pairs and stage 3 = shoot formed 3 or more leaf pairs. Dead seedlings were counted at each observation date.

Plate.3.1. Sorghum seedling in a rhizotron

3.2.2.2. Germination inducing activity of roots exudates as influenced by sorghum genotype: -

Seeds of sorghum genotypes WadAhmed, Tetron and Hakika were sterilized, germinated and the seedlings were transferred to glass tubes as 3.2.2.1 and allowed to grow hydroponically in 40% long Ashton solution for 12 days, subsequently transferred to glass tubes (100 ml capacity) wrapped with aluminum foil to exclude light and incubated for 7 days prior

to sampling. 50 ml of root exudates were taken. Samplings were made periodically at 7 days intervals. The samples (2ml from each tube) were subsequently tested for germination inducing activity. Aliquots (20µl each) were applied, each, to 8 mm glass fiber disc containing conditioned *Striga* seeds placed in Petri dishes. The seeds were re-incubated and examined for germination, haustorium initiation and radicle extension 24 h later. Seeds treated with GR24 at 0.1ppm or distilled water was included as controls for comparison.

3.2.2.3. Chromatographic behavior of root exudates fro sorghumgenotypes:-

The chromatographic behavior of germination stimulants from sorghum genotypes, WadAhmed, Tetron, and Hakika was investigated using ethyl acetate extract of root exudates obtained as in 3.2. 2.2. A sample of root exudates (100 ml) was filtrated and extracted using ethyl acetate (1:1x3), 100ml was sampled (1ml each) of the aqueous layer and organic phase layer were tested for stimulatory activity. The ethyl acetate extracts was allowed to stand over anhydrous at 4°C sodium sulphate for 24 h. The samples were filtrated and the solvent was evaporated to dryness, at 40°C, using the residue was dissolved in ethyl acetate (1 ml). Aliquots (5, 10. 15, 20, 25 and 30 µleach) of the ethyl acetate solution were applied, each, to 8 mm glass fiber discs.The discs were allowed to stand in a laminar flow cabinet for 2 h and subsequently assayed for germination inducing activity using the double disc technique (Babiker and Humdoun, 1983). Samples were assayed for germination as in 3.2.2.2**.**

3.2.2.4. Column chromatography:-

Glass chromatographic columns (34×3cm) were packed to 15 cm with silica gel (100-200 mech) obtained from s.d.FINE- CHEM IMITEd. The extract (*Ca*-0.2 ml) was loaded into the column. The column was eluted with hexane (10ml) followed in sequence by hexane: ethyl acetate mixtures

(9:1, 7:3, 1:1, 3:7, 1:9, and 0:1v/v). Fractions10ml each were collected and evaporated to dryness. The residue of each fraction was dissolved in ethylacetate (1ml). An aliquot (20µl) of each of the ethyl acetate solutions were applied, each, to 8 mm glass fiber disc. The discs were allowed to stand for 2 h and subsequently assayed for germination inducing activity using the double disc as described in 3.2.2.3

3.3. Greenhouse experiments:-

3.3.1. General:-

A series of experiments were conducted in a greenhouse at the College of Agricultural Studies (CAS), at Shambat in 2014. A soil mixture prepared by mixing Shambat soil with river sand (1:1v/v), henceforth referred to as *Striga* free soil, was used in all greenhouse experiments. Infestation of soil by *Striga hermonthica* seeds was done artificially. The artificial infestation was achieved by mixing 1g of *S. hermonthica* seeds with 1kg soil. Known weights were added to each pot to achieve the required seed bank size per pot. In all experiment sorghum seeds (5/pot) were sown at 2cm soil depth. The pots were immediately irrigated. Subsequent, irrigations were carried out every 2days. Sorghum seedlings were thinned to two plants per pot two weeks after sowing. Treatments were arranged in Randomized Complete Block Design (RCBD) with four replicates. Data collected on sorghum growth attributes were i) plant height, ii) relative leaf chlorophyll content,using a hand-held Chl meter (SPAD-502, Minolta Camera Co., Osaka, Japan) at 60 and 90 days after sowing and iiv) sorghum shoot dry weight. Striga attributes measured were, i) *Striga* emergence (plants/pot) and ii) *Striga* dry weight.

3.3.2. Effects of *Striga* seed bank size on sorghum genotypes: -

An experiment was undertaken in a greenhouse at the College of Agricultural Studies at Shambat Khartoum (Lat 22-27°N,Long 8-20°E), in

season 2014 to study plausible roles of *Striga* seed bank size and sorghum genotype in the observed spatiotemporal variability in reaction of the crop to the parasite. Three sorghum genotypes WadAhmed, Tetron and Hakika (described 3.2.1.1) were used in these experiments.

Plastic pots (30 cm i. d.), perforated at the bottom to allow for free drainage, were filled, each, to half capacity with a mix (1:1v/v) of arable soil, collected from the College farm, and river sand. *Striga* inoculum stock was prepared by mixing 1g seeds with 999 g of finely pulverized soil. *Striga* seeds inoculum was thoroughly mixed with the top 6 cm soil in each of the respective pots to achieve the required seed bank size (0-32 mg/pot). Seeds (5) of the respective sorghum genotype were subsequently planted at 2 cm soil depth. Sorghum genotypes planted in Striga-free soil were included as controls for comparison. The pots were immediately irrigated. Subsequent irrigations were carried out every 2 days. Sorghum seedlings were thinned to two plants per pot two weeks after sowing. Treatments were arranged in a Randomized Complete block Design (RCBD) with four replicates. Crop sowing, irrigation, thinning and data collection were as described in section 3.3.1. The reaction of sorghum to the parasite was assessed by counting emergent *Striga* shoots and measuring sorghum height and relative leaf chlorophyll contents using a hand-held Chl meter (SPAD-502, Minolta Camera Co., Osaka, Japan) at 60 and 90 days after sowing (DAS) and determining Striga and sorghum shoot dry weight at harvest. The reduction rate for sorghum height, RLCC and sorghum shoot dry weight for each genotype was calculated by plotting each parameter against *Striga* seed bank according to the formula

$$y = ax + c$$

Where y is the reduction rate, **x** is the *Striga* seed bank, **a** is the slope and **c** is the intercept.

3.4. Field experiments: -

3.4.1. Effects of growth stages and genotypes on stimulant production and distribution in sorghum:-

In these experiments the sorghum genotypes Wad Ahmed, Tetron and Hakika were planted in the field. Sorghum plants were sampled periodically (20, 40, 90 and 120 days after sowing). The plants, severed into shoot and roots, were dried under shade and assayed for germination inducing activity and their effects on radicle length using conditioned *Striga* seeds and the multi well sandwich methods described by Fujii, (2000).Breifly 5 ml aliquote of an agar medium prepared as described 3.2.1.6 were placed in each well of a multi-well plastic container. The agar was allowed to cool to room temperature. The plant samples to be tested (0, 10, 20, 30, 40, 50, and 60 mg/well) were evenly distributed, each in a separate well, on the agar surface. The agar was allowed to solidfy and a second 5 ml agr layer was placed on top and allowed to solidfy. Five discs containg conditioned seeds were placed on top of the second agar layer and slightly pressed to ensure contact with the agar medium. The multi-well plates, cover in place, were incubated at 30 °C for 24 h and subsequently assessed for germination as previously described in 3.2.2.2.

3.4.2. Effects of environmental conditions on the activity of stimulants from sorghum residues: -

The experiment was conducted in season 2014.The residues were dried under shade for 2 weeks and then either kept in shade.Root residues were collected directly from the field 60, 75 and 90 DAH. The roots were subsequently ground to fine powder using a kitchen grinder. Fixed weights (10, 20,30,40,50 and 60 mg/well) of the powder were assayed for germination inducing activity and haustoriu initiation as described in 3.3.3.

3.5. Statistical Analysis:-

Data collected from all experiments were subjected to statistical analysis using Statistix 8 statistical software, Version 2.0 (UK). Means were separated for significance using the Least Significant Difference (LSD at $p \geq 0.05$). Correlations were determined using GenStat (PC/Windows 7), VSN International Ltd., UK statistical package **(**Rothamsted Experimental Station). Graphs were drawn, when appropriate using Sigma plot version 11 and Microsoft Office Excel 2007.

CHAPTER FOUR

Results

4.1. Laboratory experiments: -

4.1.1. Germination, attachment and survival of *S. hermonthica* as influenced by sorghum genotyes:-

In the first week, Wad Ahmed root exudates induced 66.9% germination (Table 4.1, Appendix .1). Of the total seedlings (133.8) 66.6 % were attached. Of the attached seedlings 52.1, 28.7 and 19.2 % were at stage 1, 2 and 3, respectively. None of the seedlings was dead (Fig 4.1).Tetron root exudates induced 61.1% germination (Table 4.1, Appendix .1). Of the total seedlings (122.2) 57.7% were attached. Of the attached seedlings 66.7, 27.9 and 2.9 % were at stage 1, 2 and 3, respectively and 2.5% of the seedlings were dead (Fig4-2). Hakika root exudates induced 52.6 % germination (Table 4.1, Appendix .1). Of the total seedlings (105.2) 53.6% were attached. Of the attached seedlings 54.2, 36.3 and 6.3 % were at stage 1,2 and 3, respectively and 3.0% were dead (Fig4.3).

In the second week, cumulative germination induced by Wad Ahmed root exudates was 80.8 % (Table 4.1, Appendix .1). Of the total seedlings (161.6) 56.8% were attached. Of the attached seedlings 41.2, 35.4 and 23.4 % were at stage 1, 2 and 3, respectively and none of the seedlings was dead (Fig4.1). Cumulative germination induced by Tetron, roots exudates was72.3 % (Table 4.1, Appendix .1). Of the total seedlings (144.6) 65.9% were attached. Of the attached seedlings 51.2, 24.4 and 6.7 % were at stage 1, 2 and 3, respectively and 17.7 % were dead (Fig4-2).Cumulative germination induced by Hakika root exudates was 64.1 % (Table 4.1,

Appendix .1). Of the total seedlings (128.2) 64.5% were attached. Of the attached seedlings 45.8, 29.8 and 17.1% were at stage 1, 2 and 3, respectively and 7.2% were dead (Fig4.3). In the third week, cumulative germination induced by Wad Ahmed root exudates was 92.1% (Table 4.1, Appendix .1). Of the total seedlings (184.2) 65.8 % were attached. Of the attached seedlings 54.0, 19.9 and 24.0 % were at stage 1, 2 and 3, respectively and 1.2 % were dead (Fig4.1). Cumulative germination induced by Tetron roots exudates was 85.5 %(Table 4.1, Appendix .1). Of the total seedlings (171) 64.9% were attached. Of the attached seedlings 33.4, 29.9 and 7.1 % were at stage 1, 2 and 3, respectively and 29.6 % were dead (Fig4.2). Cumulative germination induced by Hakika roots exudates was 74.1 % (Table4.1, Appendix .1). Of the total seedlings (148.2) 68.8% were attached. Of the attached seedlings 50.2, 29.1 and 12.6 % were at stage 1, 2 and 3, respectively and 8.2 % were dead (Fig4.3).

Based on germination induced by each of the genotypes no significant differences in percentage of the seedlings in stage 1 and 2, irrespective of the time or cultivars. However, percentage seedlings at stage 3 displayed significant differences across time and genotypes. Wad Ahmed and Tetron sustained about equal percentage seedlings at stage 3 (15.3 and 14.2 %), respectively).However the percentage seedlings at stage 3was significantly lower (2.3%) on Hakika. Irrespective of genotypes, a percentage seedling at stage 3 consistently decreased with time and was 16.3, 10.3 and 5.3 % for first second and third week, respectively. Across genotypes, the lowest percentage of dead seedlings (0.4%) was displayed by those attached to Wad Ahmed, whereas, the maximum death (9.6 %) was displayed by those attached to Tetron followed by those attached Hakika. Across time percentage seedlings death was significantly lowest (0.0%) in the first week and significantly highest (7.9%) in the third week.

Table.4.1. *Striga hermonthica* germination as influenced by sorghum genotype and time in weeks post inoculation

Sorghum genotypes	Time in weeks		
	first	**second**	**third**
Hakika	52.6 (46.7) e	64.1 (53.7) cde	74.1(60.1) bcd
Tetron	61.1 (52.2) de	72.3 (59.0)bcd	85.5 (68.6) ab
Wad Ahmed	66.9 (54.9) cde	80.8 (64.4) abc	92.1 (74.3) a
Mean	**51.3 c**	**59.0 b**	**67.7 a**
SE± Cultivars		3.24	
SE± Dates		3.24	
SE± Cultivars*dates		5.62	
		F value	
Cultivars		0.0085**	
Dates		0.0002***	
Cultivars*dates		0.9594 NS	
CV		13.39	

Data within parenthesis are arcsine transformed. Data not within parenthesis are the actual germination data. ** Significant at P≤0.01 *** Significant at P≤0.001

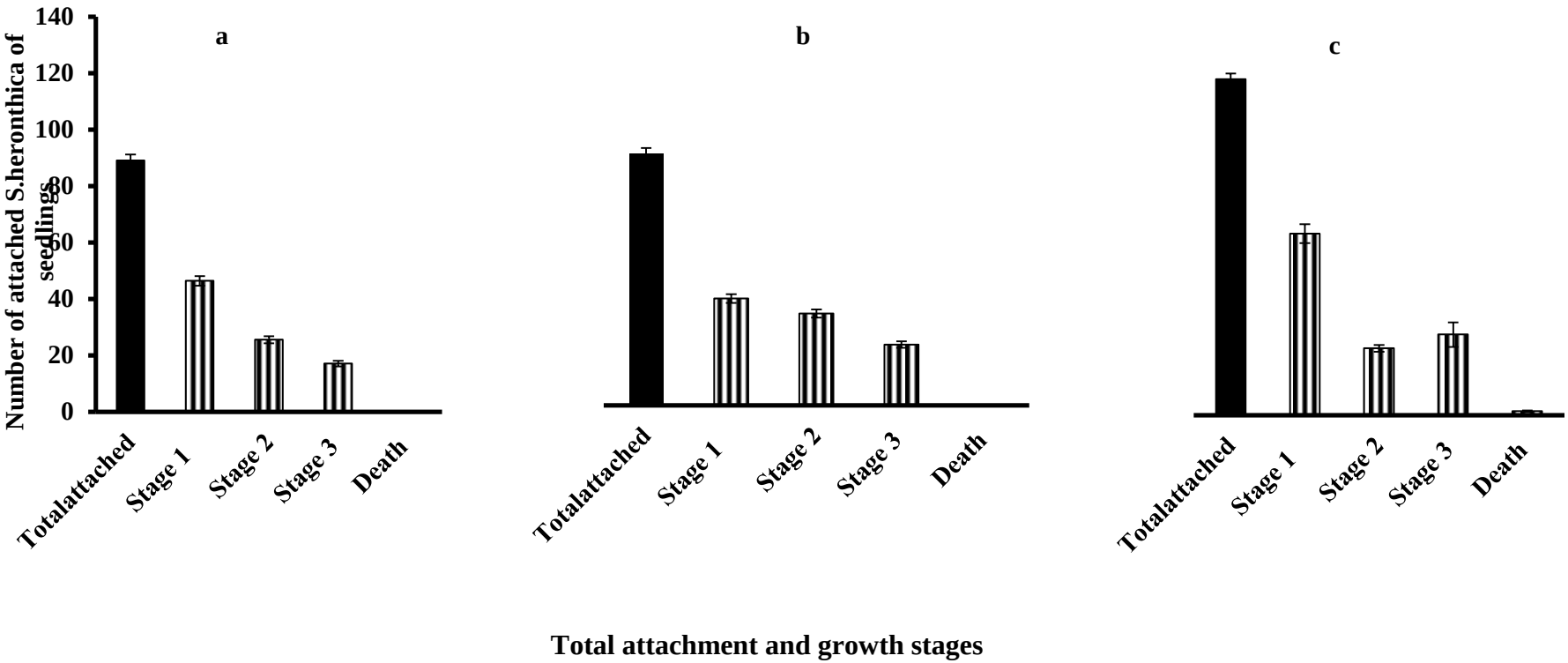

Fig.4.1. *Striga hermonthica* development and survival on **Wad Ahmed** as influenced by time. a) 7, b) 14 and c) 21 days post inoculation. Bars, each, represent a mean of 4 replicates. Vertical bars represent standard error of the means.

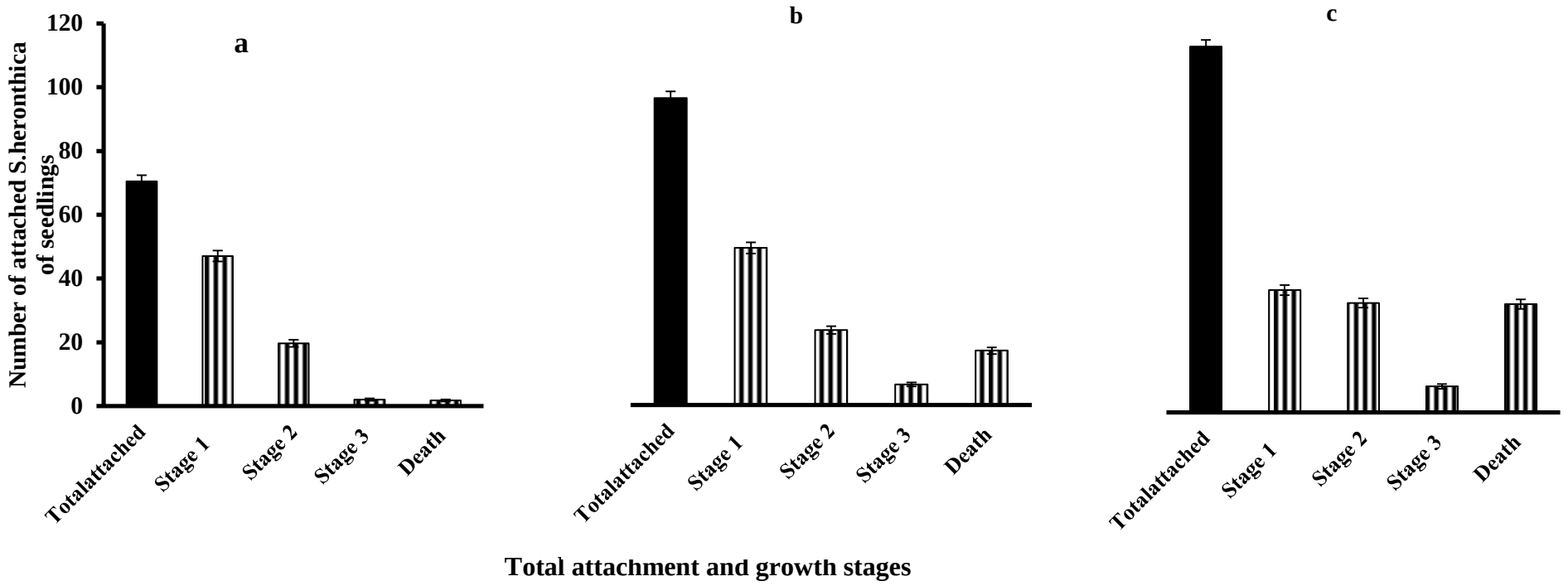

Fig .4.2. *Striga hermonthica* development and survival on **Tetron** as influenced by time. a) 7, b) 14 and c) 21 days post inoculation. Bars, each, represent a mean of 4 replicates. Vertical bars represent standard error of the means.

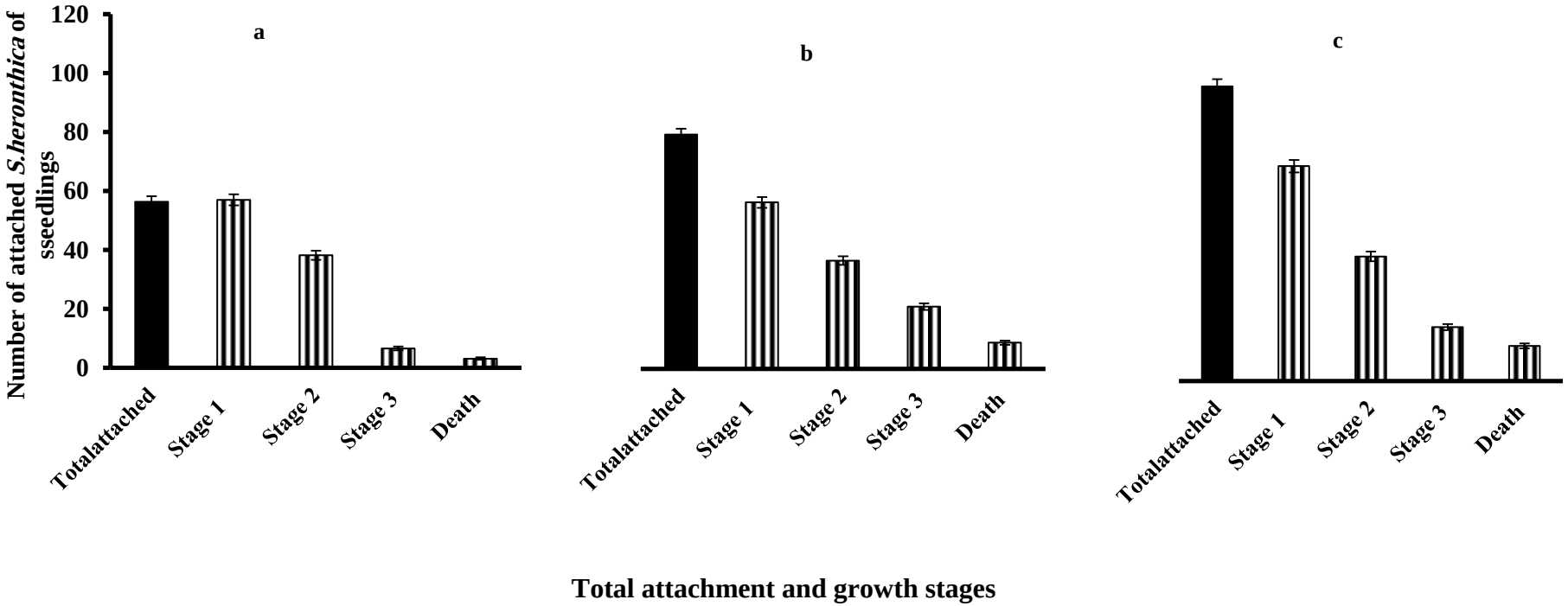

Fig .4.3. *Striga hermonthica* development and survival on **Hakika** as influenced by time. A) 7, B) 14 and C) 21 days post inoculation. Bars, each, represent a mean of 4 replicates. Vertical bars represent standard error of the means.

4.1.2. Germination inducing activity of roots exudates as influenced by sorghum genotype:-

4.1.2. 1. Effect of sorghum root exudates *on S. hermonthica* Seedlings:-

At 7 DAS, root exudates from Wad Ahmed at 5-30µl induced 27.9-39.1% germination (Table 4.2. Appendix.2).The highest and the lowest germination were attained at 5 and 30µL, respectively. For Tetron germination was7.8-15.5% with no significant differences between exudates volumes. ForHakika germination was inconsistent and varied between 19.0 and 31.6 %. Across genotypes differences in germination inducing activity of root exudates were significant. Germination was maximal (33.7%) and minimal (12.2%) for Wad-Ahmed and Tetron, respectively. Across roots exudates volumes germination was not significant and varied between 21.4-27.1% (Table 4.2.Appendix .2).

At 14 DAS, root exudates from Wad Ahmed at 5 and 10 µL levels elicited low germination (14.2 and 26.7 %, respectively). At 15-30 µL germination was 29.7-38.7% with no significant differences between treatments (Table 4.2.Appendix .2). For Tetron germination progressively increased with root exudates level, reached a peak at 20 µL (36.7%) and then, significantly, declined (Table 4.2. B). For Hakika, germination was lowest (9.5%) at 5 µL. At10µL germination increased, albeit not significantly.A further increase in root exudates volume to 15µL or more increased germination to 20.0 - 28.5% with no significant differences between treatments. Among genotypes root exudates from Wad Ahmed induced the highest germination (29.0%), while exudates from Tetron and Hakika showed significantly lower germination.For root exudates volumes germination was significantly the lowest at 5 µL (12.1%). At 10 µL a significant increase in germination was attained. Increasing root exudates volume to15-30 µL resulted in a further significant increase in germination However

differences between individual treatments were not significant (Table4.2.Appendix .2).

At 21 DAS, roots exudates from Wad Ahmed induced low germination (21.5%) at 5µL (Table4.2.Appendix .2). However, a sharp and significant increase in germination was displayed at 10 µL. Germination at 15 and20µL, though slightly depressed was at par with that at 10 µL. Increasing exudates volume to 25µL resulted in a sharp and significant increase in germination. However, at 30µL germination decreased significantly, to31.5% (Table4.2.Appendix .2). For Tetron root exudates, germination was significantly the lowest (6.5%) at 5µL.Increasing exudates volume to 10-25µL increased germination significantly. However, differences between individual levels were not significant. A further increase in root exudates volume to 30µL resulted in a further significant increase in germination. For Hakika, germination tended to be low (26.2-30.9%) at 5-15 µL with no significant differences between individual root volumes (Table4.2.Appendix .2). Increasing root exudates volume to 20-30µl increased germination significantly, with no further significant differences between individual exudates volume (Table4.2.Appendix.2). Among genotypes root exudate from Wad-Ahmed and Hakika induced the highest germination 39.1 and 36.8 %, respectively, while root exudates from Tetron affected significantly lower germination. For root exudates volumes germination progressively increased with exudates volume. Germination was lowest (19.64%) at 5 µL. Increasing root exudates to 10 and 20 µL increased germination significantly, with no significant differences between individual treatments. Increasing root exudates volume to 25 µL increased germination in comparison to 20 µL, albeit not significantly. A further increase in root exudates volume to 30 µL resulted in the highest germination (40.8%) which was at par with its congener observed at 25µL (Table 4.2.Appendix .2).

At 28 DAS, root exudates from Wad Ahmed induced inconsistent germination that ranged between 17.6-33.3% with no significant differences between individual volumes (Table4.2.Appendix .2). Root exudates from Tetroninduced variable and inconsistent germination which showed no significant differences between exudates volumes. Root exudates from Hakika induced little germination reaching a maximum (26.23%) at 15µL with no further increase with exudates volume (Table4.2. Appendix .2).Among genotypes root exudates from Tetron induced the highest germination (34.7%). Root exudates from Wad-Ahmed and Hakika displayed comparable germination inducing activity which was significantly lower than that of Tetron. For root exudates volumes, irrespective of genotype, germination ranged between 24.7 and 30.8 % with no significant difference (Table4.2.Appendix.2).

Table. 4.2. Effects of hydroponically grown sorghum genotypes root exudates on germination of *S.hermonthica* Seeds:-

Days
Germination %
Root exudates volume(µL)
7DAS

Cultivars	5	10	1 5	20	25	30	Mean
Wad Ahmed	39.1 a	29.5 abc	39.0 a	32.3 abc	34.6 ab	28.0 bcd	**33.8 a**
Tetron	11.6 fg	15.5 efg	7.8 g	15.3 efg	10.0 fg	12.8 fg	**12.2 c**
Hakika	30.5 abc	19.0 def	30.2 abc	23.6 cde	30.5 abc	31.6 abc	**27.5 b**
Mean	**27.1 a**	**21.4 a**	**25.6 a**	**23.7 a**	**25.0 a**	**24.1 a**	
			14 DAS				
Wad Ahmed	14.2 ghi	26.7 cdef	30.7 abcd	34.1 abc	29.7 abcd	38.7 a	**29.0 a**
Tetron	12.2 hi	14.1 ghi	25.1 cdef	36.7 ab	25.2 cdef	24.3 def	**23.0 b**
Hakika	9.6 i	18.0 fghi	23.0 defg	20.0 efgh	26.1 cdef	28.5 bcde	**20.8 b**
Mean	**12.1 c**	**19.6 b**	**26.3 a**	**30.2 a**	**27.0 a**	**30.5 a**	
			21 DAS				
Wad Ahmed	21.5 g	44.6 bc	39.4 cde	37.4 cdef	60.1 a	31.5 defg	**39.1 a**
Tetron	6.5 h	20.4 g	23.9 g	18.8 gh	20.6 g	38.6 cdef	**21.5 b**
Hakika	30.9 defg	26.2 fg	28.4 efg	42.9 bcd	40.1 bcde	52.4 ab	**36.8 a**
Mean	**19.6 d**	**30.4 c**	**30.6 c**	**32.1 bc**	**40.3 ab**	**40.8 a**	
			28 DAS				
Wad Ahmed	21.7 cde	27.0 abcde	27.0 abcde	27.0 abcde	17.6 de	33.3 abc	**25.6 b**
Tetron	41.1 a	26.3 abcde	38.4 ab	39.5 ab	30.4 abcde	32.4 abcd	**34.7 a**
Hakika	14.8 e	23.1 cde	26.2 abcde	26.0 abcde	26.0 abcde	24.4 bcde	**23.4 b**
Mean	**25.9 a**	**25.4 a**	**30.5 a**	**30.8 a**	**24.7 a**	**30.0 a**	

Means within a rowand a column followed by the same letter(s) are not significantly different according to LSD at 5%.

4.1.2.2. Effects of root exudates on radicle length: -

At 7DAS, radicle length, in response to root exudates from Wad Ahmed increased with volume reached a peak at 20 µL and then declined significantly (Table4.3.Appendix.3). For Tetron root exudates, radicle length, with single exception, showed no significant change at exudatesvolumes of 5-20 µL. However, a non significant decline occurred at 25µL. A further increase in root exudates volume to 30 µL resulted in a significant decrease. For root exudates from Hakika the highest radicle length was displayed at the lowest root exudates volume (5 µL). A further increase in root exudates volume to 10µL resulted in non-significant decrease in radicle length. Subsequent increase in exudates volume to 15-30 µL resulted in significant decrease in radicle length (Table4.3. Appendix .3).Across genotypes Wad Ahmed and Hakika showed comparable radicle length. However, for Tetron radicle length was significantly lower. Across exudates levels, with single exception, radicle length was maximal at 20 µL and a non-significant decline in radicle length occurred at 30µL exudates volume (Table 4.3. Appendix .3)

At 14 DAS, for root exudates from Wad Ahmed radicle length was maximal at 10 µL. A consistent and significant decline in radicle length was displayed on further increase in exudates volume from 15 to 30µL (Table 4.3.Appendix .3). For Tetron root exudates, radicle length was maximal at 10µL.A further increase in root exudates to 15µL or more, with a single exception at 25µL, showed consistent and significant suppression of radiclelength (Table4.3. Appendix.3). For root exudates from Hakika radicle length was maximal at 10µL. At 15 exudates volume a non - significant decrease in radicle length was displayed. Subsequent increase of exudates volume to 20-30, with a single exception, resulted in significant decline in radicle length (Table4.3.Appendix .3). Across genotypes radicle

length showed no significant differences. A cross exudates volumes radicle length was maximal at 10µL. A further increase in exudates volumes to 15-30µLwith a single exception, resulted in significant decrease in radicle length (Table 4.3. Appendix .3)

At 21 DAS, for root exudate from Wad Ahmed radicle length was maximal at 5µL. Subsequent increase in exudates volume to 10-30µL resulted in consistent, albeit non-significant decline in radicle length. For root exudates from Tetron, radicle length was maximal (4.6µm $\times 10^{-2}$) at 25 with no significant differences between all treatments (Table4.3.Appendix.3). For Hakika root exudates affected maximal radicle length at 5µL. Increasing exudates volume up to 25µL decreased radicle length albeit non-significantly. However a further increase in exudates volume to 30 µL resulted in a significant decline (Table4.3.Appendix .3). Across genotypes root exudates from Hakika showed the highest radicle length (4.5µm $\times 10^{2}$). Root exudates from Tetron sustained comparable radicle length however; root exudates from Wad Ahmed yielded significantly lower radicle length. For exudates levels radicle length was maximal (4.4µm $\times 10^{-2}$) at the lowest level (5µl). Increasing exudates volume to 10-30 µL resulted in non-significant decrease (Table4. 3. Appendix .3)

At 28 DAS, for root exudates from Wad Ahmed radicle length was more or less constant (2 µm $\times 10^{-2}$) up to 20µL (Table 4.3.Appendix .3). However, non-significant increments in radicle length were displayed on further increase in root exudates volume. For Tetron radicle length was maximal and significant (3.5 µm $\times 10^{-2}$) at exudates volume of 10µL. However, further increase in exudates volume resulted in significant decline. For roots exudates from Hakika radicle length was maximal (4.0µm $\times 10^{-2}$) at 5µL exudates level. A further, increase in exudates volume to 15µL decreased radicle length, albeit not significantly. However,

radicle length showed significant decrease on further increase in exudates volume with no significant differences (Table4.3.Appendix .3).

Across genotypes radicle length was significantly maximal (2.9 µm×10^{-2}) for Tetron root exudates and minimal (0.3 µm×10^{-2}) for Wad Ahmed. Across exudates volumes radicle length was significantly maximal at 5 and10. However, a further increase in exudates volume to15µL or more decreased radicle length significantly (Table 4.3.Appendix .3).

Table.4.3. Effects of hydroponically grown sorghum genotypes root exudates on radicle length:-

Days

Radicle ($\mu m \times 10^{-2}$)

Root exudates volume (µL)

Cultivars	5	10	1 5	20	25	30	Mean
			7DAS				
Wad Ahmed	0.9 e	1.0 de	1.3 cd	1.9 a	1.25 cd	1.00 de	**1.21 a**
Tetron	1.0 de	1.3 cd	0.4 f	1.0 de	0.68 ef	0.35 f	**0.78 b**
Hakika	1.6 ab	1.5 bc	0.9 e	1.0 de	1.00 de	1.00 de	**1.17 a**
Mean	**1.2 ab**	**1.3 a**	**0.9 c**	**1.3 a**	**1.0 bc**	**0.8 c**	
			14 DAS				
Wad Ahmed	2.9 abc	3.4 a	2.4 bcde	2.3 bcde	2.3 bcde	1.6 e	**2.46 a**
Tetron	2.6 abcd	3.1 ab	2.6 abcd	2.3 bcde	2.9 abc	1.6 e	**2.52 a**
Hakika	2.8 abcd	3.1 ab	2.0 cde	1.9 de	3.0 ab	2.0 cde	**2.46 a**
Mean	**2.8 ab**	**3.2 a**	**2.3 bc**	**2.1 cd**	**2.7 ab**	**1.8 d**	
			21 DAS				
Wad Ahmed	4.1 abc	3.9 bc	3.9 bc	3.6 bc	3.4 c	3.6 bc	**3.73 b**
Tetron	3.5 bc	4.0 bc	4.1 a bc	3.8 bc	4.6 abc	4.0 bc	**4. 00 ab**
Hakika	5.5 a	4. 6 abc	4.9 ab	4.3 abc	4.5 abc	3.5 bc	**4.54 a**
Mean	**4.4 a**	**4.1 a**	**4.3 a**	**3.9 a**	**4.2 a**	**3.7 bc**	
			28 DAS				
Wad Ahmed	0.2 g	0.2 g	0.2 g	0.2 g	0.3 g	0.8 fg	**0.31 c**
Tetron	2.3 cd	3.5 a	1.5 def	2.0 cd	1.6 de	1.0 efg	**2.89 b**
Hakika	4.0 a	3.8 a	3.3 ab	2.6 bc	1.8 de	2.0 cd	**1.97 a**
Mean	**2.2 a**	**2.5 a**	**1.7 b**	**1.7 b**	**1.2 b**	**1.3 b**	

Means within a row a columnfollowed by the same letter(s) are not significantly different according to LSD at 5%.

4.1.2.3. Effects of root exudates on Haustorium:-

At 7 DAS, irrespective, of sorghum genotype or root exudates volume, haustorium induction was low (1.2–4%) and inconsistent (Fig.4.4, Appendix .4). At 14 DAS, root exudates from Wad Ahmed induced 11.9-33.6% haustorium initiation; however differences between exudates volumes were not significant. The lowest and highest haustorium initiations were displayed at root exudates volumes of 15 and 30 µL (Fig.4.4, Appendix .4). For Tetron root exudates, haustorium initiation progressively increased with root exudates volume from 5 to 15 µL. However, differences were not significant. A further increase in volume up to 30 µL resulted in slight, albeit significant; drop in haustorium formation (Fig.4.4, Appendix .4). Root exudates from Hakika initiated very low haustorium induction which ranged between 0.0 - 8.7 % with no significant differences between volumes. Among genotypes root exudates from Tetron and Wad Ahmed showed the highest haustorium initiation, while Hakika showed the lowest (Fig.4.4, Appendix .4). A cross root exudates volumes haustorium initiation showed no significant differences.

At 21 DAS, root exudates from Wad Ahmed at its lowest levels (5 and 10 µL) induced low haustorium initiation (23.0 and 16.9%, respectively).However, increasing exudates levels to 20µL or more increased haustorium initiation significantly (Fig.4.4.B, Appendix .4). Root exudates from Tetron and Hakika, irrespective of volume induced negligible haustorium initiation (0.0- 8.3 %). Among varieties root exudates from Wad-Ahmed induced significantly, the highest haustorium initiation (31.7 %). Root exudates from (< 2 %). For root exudates volumes, with a single exception, no significant differences were realized (Fig.4.4, Appendix .4).

At 28 DAS, root exudates from Wad Ahmed induced high haustorium initiation (> 90 %). Root exudates from Tetron displayed low haustorium

initiation (18.1%) at its lowest levels. Increasing root exudates volume, in general, resulted in decreased haustorium initiation. At the highest root exudates volume (30µL) haustorium initiation dropped to 0.0 %. Root exudates from Hakika irrespective of level, induced negligible haustorium initiation (0-3%). Among genotypes root exudates from Wad Ahmed showed significantly the highest haustorium initiation (94.2 %), while root exudates from Hakika showed significantly the lowest haustorium initiation (< 1%) (Fig.4.4, Appendix .4).

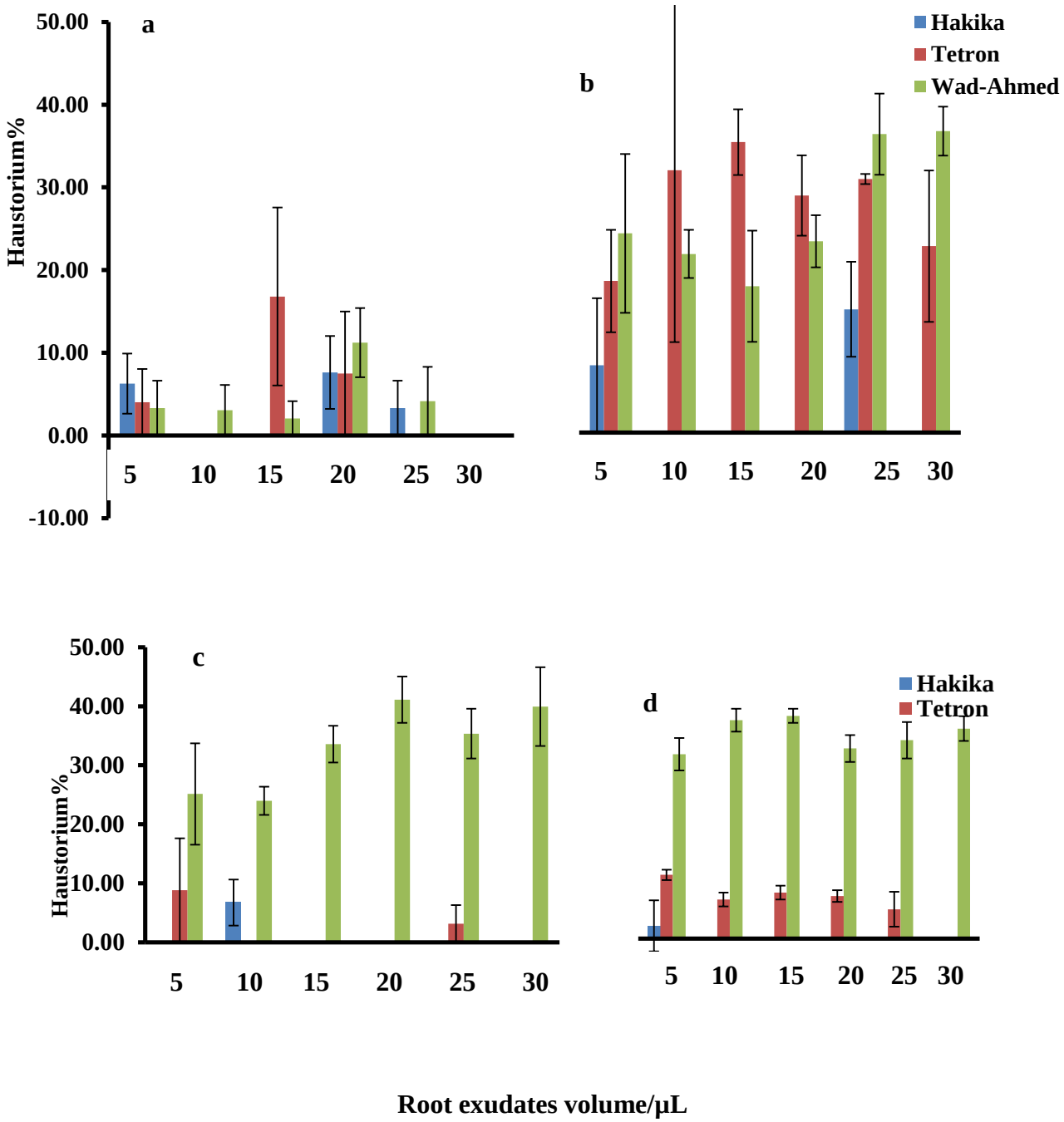

Fig. 4. 4. Effects of root exudates of hydroponically grown sorghum on haustorium initiation of *S. hermonthica* a) 7 DAS, b) 14 DAS, c) 21 DAS and d) 28 DAS. Vertical represented stander error.

4.2. Greenhouse experiments:-

4.2.1. Effects of *Striga* seed bank size on sorghum genotypes:-

4.2.1.1. Effects on *Striga*:-

4.2.1.1.1. Emergence:-

S. hermonthica emergence progressively increased with seed bank size, but varied in magnitude with sorghum genotype (Fig. 4.5.a and b). Among the genotypes Wad Ahmed sustained the highest *Striga* emergence followed in descending order by Tetron and Hakika.

At 60 DAS, *Striga* emergence on Wad Ahmed was lowest (2.8 plants/pot) at the lowest seed bank size (2 mg/pot). Increasing seed bank size to 4 and 6 mg/pot increased *Striga* emergence, albeit not significantly. A further increase in *Striga* seed bank size to 8 mg/pot or more increased *Striga* emergence significantly (Fig.4.5.a). Emergence of the parasite was 7.0, 7.0, 11.0 and 12.5plants/pot at seed bank size of 8, 12, 16 and 32 mg/pot, respectively. On Tetron, *Striga* emergence at low seed bank size (2- 6 mg/pot) was 1.5-3.3plants/pot. Increasing seed bank size to 8 and 12 mg/pot increased *Striga* emergence to 4.5 and 4.3 plants/pot, respectively. A further increase of the parasite seed bank size to 16 and 32 mg/pot increased emergence to 5.8 and 8.5plants/pot, respectively (Fig.4.5.a).On Hakika, *Striga* emergence at low seed bank size (2-6 mg/pot) was 0.5-1.0 plants/pot. Increasing seed bank size to 8-12 mg/pot did not affect a significant increase in *Striga* emergence. However, increasing *Striga* seed bank size to 16 and 32 mg/pot increased *Striga* emergence significantly to 3.3 and 4.8 plants/pot, (Fig. 4.5.a). Striga emergence was highly and positively correlated with seed bank size.The correlation coefficient (r) was0.73, $P \leq 0.01$, 0.82, $P \leq 0.01$ and 0.83, $P \leq 0.01$ for Wad Ahmed, Tetron and Hakika, respectively.

At 90 DAS, *Striga* emergence at low seed bank size (2 - 6 mg/pot) on Wad Ahmed was 7.8-8.3 plants/pot. Increasing seed bank size to 8 and 12

mg/pot, increased *Striga* emergences to 14.5 and 16.3 plants/ pot, respectively.A further increase in *Striga* seed bank to 16 and 32 mg/pot increased *Striga* emergence to 20.8 and 23.0 plants/pot, respectively (Fig. 4.5.b). On Tetron*Striga* emergence at seed banks of 2-6 mg/pot was 1-2.5 plants/pot. A further increase in seed bank size to 8-12 and 16-32 mg/pot increased *Striga* emergence to 8.8-9.3 and 8.5-10.5, plants/pot, respectively. (Fig.4.5.b). On Hakika *Striga* emergence was 2.0-4.8 plants/pot with no significant differences between seed bank sizes (Fig.4.5.b). *Striga* emergence showed high and positive correlation with *Striga* seed bank on Wad Ahmed ($r = 0.66$, $P \leq 0.01$) and Tetron ($r = 0.7$, $P \leq 0.01$), but only a moderate correlation ($r = 0.44$, $P \leq 0.01$) was displayed on Hakika.

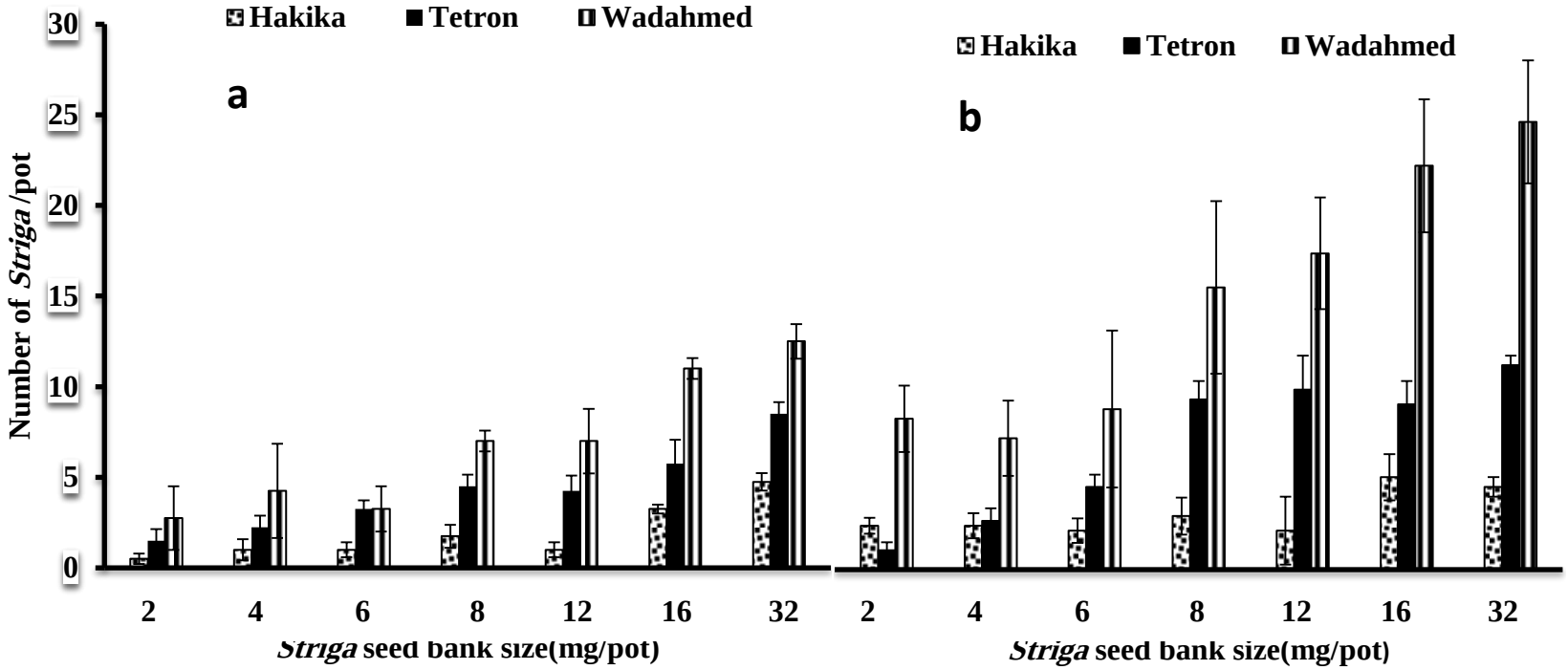

Fig4.5. Effects of *Striga* seed bank size on *Striga* emergence, (a) 60 DAS, (b) 90 DAS. The vertical bar represents standard error

4.2.1.1.2. Effects on *Striga* dry weight:-

Wad Ahmed sustained the highest *Striga* dry weight followed in descending order by Tetron and Hakika **(**Table 4.4). *Striga* dry weight on Wad Ahmed consistently increased with seed bank size **(**Table4.4). At a seed bank size of 2-6 mg/pot, Striga dry weight was 1.5-3 g/pot. An increase in *Striga* seed bank size to 8 mg/pot increased *Striga* dry weight to 5.3 g/pot and the attained increment was significant. A further increase in *Striga* seed bank to 12 mg/pot increased the parasite dry weight, albeit not significantly. However, at a seed bank size of 16 mg/pot a significant increase in dry weight was attained. Increasing *Striga* seed bank size to 32 mg/pot resulted in a further significant increase in dry weight.On Tetron at the lowest seed bank size (2 mg/pot), *Striga* dry weight showed an average of 2.5 g/pot **(**Table4.4).Increasing seed bank size to 4 mg/pot increased dry weight significantly. A further increase in seed bank size to 6-16 mg/pot increased *Striga* dry weight considerably, albeit not significantly. A further increase in seed bank size to 32 mg/pot, on the other hand, resulted in a significant increase in dry weight. On Hakika *Striga* seed bank at 2-12 mg/pot affected a dry weight of 0.8- 2.5g/pot with no distinct trends and no significant differences between tratments. At a seed bank of 16 mg/pot, however, a significant increase in *Striga* dry weight was attained. Afurther increase in *Striga* seed bank to 32 mg/pot resulted in a further significant increase in dry weight **(**Table4.4).Striga dry weight was positively correlated with Striga seed bank. The correlation coefficient r was 0.92, $P \leq 0.01$, 0.78, $P \leq 0.0$ and 0.85, $P \leq 0.01$) for Wad Ahmed, Tetron and Hakika, respectively.

Table.4.4. *Striga* dry weight as influenced by sorghum genotypes and seed bank size:-

	***Striga* dry weight (g)**		
	Sorghum genotypes		
***Striga* seed bank size (mg)**	**Wad Ahmed**	**Tetron**	**Hakika**
2	1.5 d	2.5 c	0.8 c
4	2.5 cd	4.5 bc	1.3 c
6	3.0 cd	4.3 bc	1.5 c
8	5.3 c	4.0 bc	1.5 c
12	5.8 bc	5.3 bc	2.5 c
16	9.0 b	6.3 b	4.5 b
32	17.5 a	9.8 a	7.3 a

Means within a row or acolumn followed by the same letter(s) are not signific different according to LSD at 5%.

4.2.1.2. Effects on Sorghum genotype:-

4.2.1.2.1. Plant height (cm): -

Reduction in sorghum height, irrespective of genotype or time, progressively increased with seed bank size (Table 4.5).In general at 60 DAS Wad Ahmed displayed low, none significant reductions in height, irrespective of seed bank size and the maximal reduction (24.7 %) was attained at the highest seed bank size (Table 4.5). InTetron and Hakika *Striga* seed bank size at 2-16 mg/pot affected non-significant reductions in height (2.9 -16.5%). However, at the highest seed bank size (32 mg/pot) significant reductions of 29.7% and 9.2% were displayed by Tetron and Hakika, respectively. The correlation coefficient was very low for Hakika ($r = -0.24$, $P \leq 0.01$), low for Wad Ahmed (r -0.33, $P \leq 0.01$) and moderate for Tetron ($r = -0.40$, $P \leq 0.01$). Reduction rates in height were low for Wad Ahmed (0.31, $P \leq 0.01$), Tetron (0.41, $P \leq 0.01$) and Hakika (0.19, $P \leq 0.01$).

At 90 DAS Wad Ahmed displayed non-significant reduction (4.9 %) in height at the lowest seed bank size (2mg/pot) (Table4.5). Increasing seed bank size to 4mg/pot or more affected significant reductions which consistently increased with seed bank size reaching a peak (39.6 % reduction) at the highest seed bank size (32mg/pot). For Tetron *Striga* seed bank at 2 and 4mg/pot affected insignificant reductions in height (9.5 and 9.9%, respectively). Increasing seed bank size to 6 mg/pot or more resulted in significant reductions which consistently increased with seed bank and was maximal (37.7 and 36.3%) at the seed bank size of 16 and 32 mg/pot, respectively. In Hakika *Striga* seed bank at 2 mg per pot affected insignificant drop (2.8 %) in plant height. However, increasing seed bank size to 4 mg/pot or more, increased height significantly with no significant differences between levels. Plant height was negatively correlated with Striga seed bank size. The correlation coefficients were high for Wad Ahmed ($r = 0.81$, $P \leq 0.01$), moderate 0.68, $P \leq 0.01$) for Tetron and very low for Hakika -0.15) $P \leq 0.01$). The reduction rates were 1.01 ($P \leq 0.01$) for Wad Ahmed, 0.95 ($P \leq 0.01$) for Tetron and 0.19 ($P \leq 0.01$) for Hakika (Table4.5).

Table .4.5. Sorghum height as influenced by genotype and *Striga* seed bank size:-

	Days after sowing							
	60 DAS							
	Striga seed bank size (mg/ pot)							
Cultivars	**0**	**2**	**4**	**6**	**8**	**12**	**16**	**32**
Wad Ahmed	44.5 a	44.5 a	41.3 a	41.6 a	43.1 a	43.1 a	40.7 a	33.5 a
Tetron	51.6 a	43.1 ab	50.1 ab	50.1 ab	46.0 ab	44.6 ab	43.5 ab	36.3 b
Hakika	42.4 ab	42.8 ab	46.3 a	50.4 a	48.0 ab	49.3 ab	42.1 ab	38.5 b
LSD of levels	7.5							
LSD of culltivars	4.6							
LSD of level* culltivars	13.0							
CV %	20.9							
	90 DAS							
Wad Ahmed	83.6 a	79.5 a	71.6 b	67.1 bc	63.6 c	61.9 c	51.1 d	50.5 d
Tetron	88.0 a	79.6 ab	79.3 ab	73.8 bc	61.1 cd	70.0 bc	54.8 d	56.1 d
Hakika	61.5 b	59.8 b	78.1 a	80.3 a	79.3 a	80.4 a	78.3 a	70.5 ab
LSD of levels	7.1							
LSD of culltivars	4.3							
LSD of level* culltivars	12.2							
CV %	12.4							

Means within arrow or a column followed by the same letter(s) are not significantly different a ccording to LSD at 5%.

4.2.1.2.2. Relative leaf chlorophyll content (SPAD-value):-

SPAD-502 values showed that at 60 and 90 DAS, across genotpes, RLCC consistently decreased with increasing seed bank size for Wad Ahmed, however, for Tetron and Hakikaa high degree of inconsistency was displayed (Table4.6). At 60 DAS *Striga* seed bank size of 2 and 4 mg/pot affected insignificant reductions (<20%) in RLCC in Wad Ahmed. Increasing seed bank size to 6 mg/pot or more resulted in significant reductions (26.3-40.4 %). Reductions in RLCC in Wad Ahmed were highly and negatively correlated with seed bank size ($r = -0.665$, $P \leq 0.01$). Reductions in RLCC in Tetron and Hakika were not significant and displayed low negative correlation with seed bank size ($r = -0.008$ and -0.093, $P \leq 0.01$, respectively). The reduction rate was 0.345 ($P \leq 0.01$) for Wad Ahmed, 0.005 ($P \leq 0.01$) for Tetron and 0.049 ($P \leq 0.01$) for Hakika (Table4.6).

At 90 DAS Wad Ahmed displayed non-significant reduction (9.8 %) in RLCC at the lowest *Striga* seed bank size. Increasing seed bank size to 4-12 mg/pot or more affected progressive and significant reductions in RLCC (Table4.6). At seed bank size of 16 and 32 mg/pot, a sharp decline (53 and 61.7%, respectively) in RLCC was realized. In Tetron RLCC showed inconsistent and insignificant reductions (17.2 and 9.7%) at seed bank sizes of 2 and 4 mg/pot. Increasing seed bank size to 6-12 mg/pot resulted in significant reductions (37.5-39.1%) in RLCC. A further increase in *Striga* seed bank size showed inconsistent response. In Hakika the RLCC, with a single exception, displayed insignificant reductions with increasing seed bank size. Reductions in RLCC in Wad-Ahmed were highly and negatively correlated with seed bank size ($r = -0.73$ $P \leq 0.01$). Reductions in RLCC in Tetron and Hakika were not significant and displayed low negative correlation with seed bank size (-0.238**,** $P \leq 0.01$and -0.236, $P \leq 0.01$, respectively). The reduction rate was 0.71 ($P \leq 0.01$) for Wad Ahmed, 0.18 ($P \leq 0.01$) for Tetron and 0.25 ($P \leq 0.01$) for Hakika (Table4.6).

Table.4.6.Sorghum relative leaf chlorophyll content (SPAD-value) as influenced by genotype and *Striga* seed bank size:-

	Days after sowing							
	60 DAS							
	Striga seed bank size (mg /pot)							
Genotypes	**0**	**2**	**4**	**6**	**8**	**12**	**16**	**32**
Wad-Ahmed	31.2 a	26.7 ab	27.0 ab	22.8 bc	23.0 bc	21.4 c	19.2 c	18.6 c
Tetron	30.6 a	26.7 a	28.6 a	33.4 a	31.3 a	33.2 a	27.0 a	28.3 a
Hakika	25.8 ab	25.3 ab	26.1 ab	25.7 ab	22.3 ab	21.2 b	29.9 a	24.8 ab
LSD of levels			4.0					
LSD of culltivars			2.4					
LSD of level* culltivars			6.9					
CV %			18.5					
	90 DAS							
Wad-Ahmed	38.9 a	35.1 abc	9.9 bc	5.9 cd	8.7 cd	22.4 de	8.3 ef	14.9 f
Tetron	32.0 a	26.5 abc	8.9 ab	0.0 bc	20.7 bc	19.5 c	3.9 abc	23.4 abc
Hakika	38.5 a	35.0 ab	28.8 ab	27.3 ab	27.4 ab	28.7 ab	22.0 b	28.6 ab
LSD of levels			6.4					
LSD of culltivars			3.9					
LSD of level* culltivars			11.1					
CV %			29.4					

Means with arow or a column followed by the same letter(s) are not significantly different according to LSD at 5%.

4.2.1.2.3. Effects on Sorghum dry weight:-

Striga, irrespective of seed bank size inflicted significant reductions in dry weight of Wad Ahmed. At *Striga* seed bank size of 2 mg/pot a sharp reduction (50.8%) in dry weight was displayed (Fig.4.6). Increasing seed bank to 4-8 mg/pot resulted in further reductions in dry weight, albeit not significantly. Increasing seed bank size to 12-32 mg/pot inflicted further significant reductions amounting to 88.2-89.5%with no significant differences between seed banks levels (Table 4.7).

The dry weight of Wad Ahmed was moderately and negatively correlated with *Striga* seed bank (r= -0.59, P≤ 0.01) (Table 4.7). For Tetron *Striga* seed bank of 2-6 mg/pot inflicted low and insignificant reductions in dry weight (13.6-23.1%). Increasing *Striga* seed bank to 8 and 12 mg/pot resulted in significant reductions in comparison to the *Striga*-free control (Fig.4.6). However, the attained dry weights were statistically at par with those obtained at the seed banks of 4 and 6 mg/pot. A further increase in Striga seed bank to16 and 32 mg/pot significantly depressed dry weight, but the dry weights obtained were at par with that attained at the seed bank size of 12 mg/pot. The dry weight of Tetron was highly and negatively correlated with *Striga* seed bank size (r= -0.73, P≤ 0.01). For Hakika a slight insignificant increase in dry weight (18.4%) was achieved at a seed bank size of 2 mg/pot. Increasing seed bank size to 4 mg/pot resulted in a sharp significant reduction (45.5%) in dry weight (Fig.4.6). Further increase in *Striga* seed bank size resulted in further reductions which, with a single exception, progressively increased with the parasite seed bank size reaching a peak (83.1%) at the highest seed bank size (Table 4.7). However, Differences between treatments were not significant. The dry weight of Hakika showed a moderate and negative correlation with seed bank size (r = -0.60, P≤ 0.01). The reduction rate was 1.90 (P≤ 0.01) for Wad Ahmed, 1.87(P≤ 0.01) for Tetron and 1.74 (P≤ 0.01) for Hakika.

Table.4.7. Effect of *striga* seed bank size on dry weight of sorghum genotype:-

	Sorghum dry weight (g)					
	Genotype					
***Striga* seed bank size(mg)**	**Wad Ahmed**		**Tetron**		**Hakika**	
0	93.1	a	89.9	a	65.1	ab
2	44.9	b	77.6	a	77.1	a
4	41.5	b	67.9	ab	35.5	bcd
6	32.4	bc	69.1	ab	41.4	bc
8	28.8	bc	52.0	b	16.8	cd
12	9.8	c	47.6	bc	26.4	cd
16	11.0	c	28.8	c	14.9	cd
32	10.3	c	29.5	c	11.0	d
LSD of levels	14.4					
LSD of culltivars	8.8					
LSD of level* culltivars	25.0					
CV %	41.5					

Means with a row or a column followed by the same letter(s) are not significantly different according to LSD at 5%.

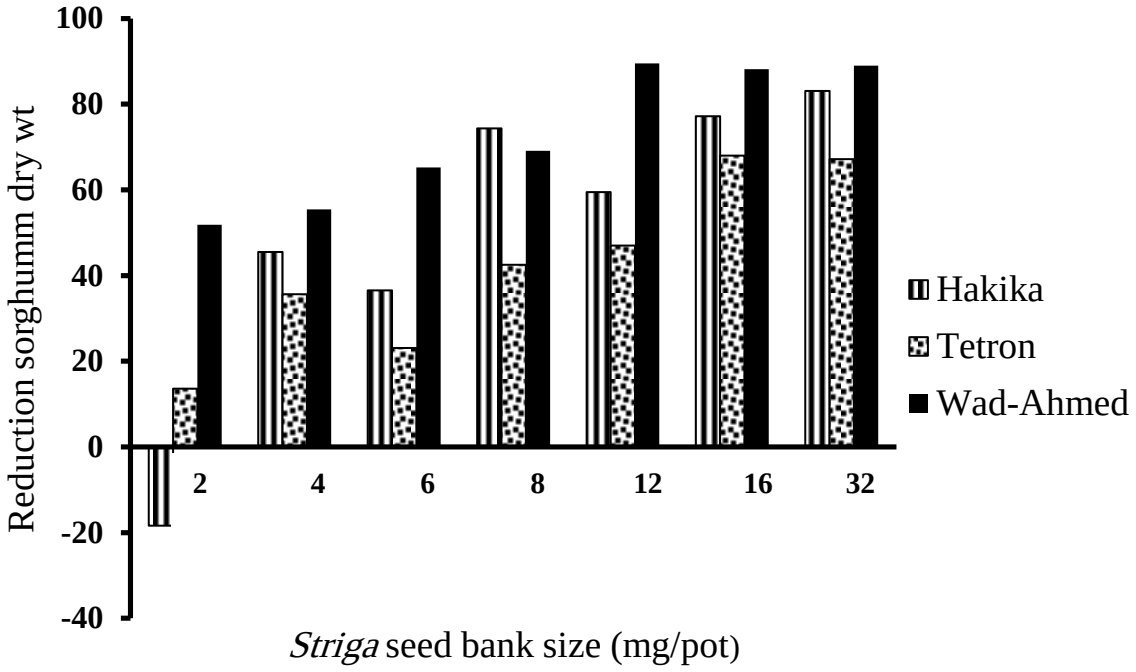

Fig.4.6. Effect of *Striga hermonthica* seed bank size on Sorghum dry weight.

4.3. Field experiments:-

4.3.1. Effects of growth stages and genotypes on stimulant production and distribution in sorghum: -

4.3.1.1. Effect on germination:-

4.3.1.1. 1. Germination inducing activity of shoot powder: -

Sorghum powder, irrespective of genotype or plant parts, sampled periodically induced germination of *S.hermonthica* seeds. However, the response showed dependence on genotype, powder amount and the time of collection.

At 20 DAS, Wad Ahmed shoot powder at 10 and 20 mg/well induced the highest germination (74.8%). However, germination progressively decreased with increasing amount of powder. At 30mg/well germination decreased, albeit not significantly.A further increase in shoot powder to 40 mg/well or more decreased germination significantly (Fig4.7.a, Appendix .5).Germination response to shoot powder from Tetron showed similar trends (Fig4.7.a). At 10 mg/well shoot powder induced 31% germination. Increasing powder amount to20 mg/well increased germination to 58%. Increasing powder amount to 30 mg/well resulted in a slight non-significant increase in germination (60%). On further increase of the powder to 40 and 50 mg/well a gradual, albeit non-significant, decline in germination inducing activity was observed (Fig4.7.a, Appendix .5). For Hakika germination inducing activity showed an inconsistent trend (Fig4.7.A, Appendix .5). At 10 mg/well germination was 34%. At 20 mg/well germination increased to 37%. Increasing shoot powder to 30 mg/well decreased germination. However, germination inducing activity was comparable at all powder amounts (10-50mg/well) (Fig4.7.a, Appendix .5).

At 40 DAS, in general, germination inducing activity was higher than at 20 DAS, Wad Ahmed shoot powder at 10 mg/well displayed very high

germination inducing activity (89%). However, germination at 20 mg/well decreased to 49 %. A further increase in amount of shoot powder to 30-50 mg/well resulted in slight non significant decrease in germination (Fig4.7.b). Tetron shoot powder at 10 and 20 mg/well induced poor germination (< 40%). However, increasing shoot powder amount to 30 and 40 mg/well increased germination to 51 and 61%, respectively. A further, increase in amount of powder to 50 mg/well decreased germination to 46 %, respectively. Hakika shoot powder at 10 mg/well induced 39% germination (Fig4.7.b, Appendix .5). Increasing shoot powder up to 40 mg/well decreased germination, albeit not significantly. At 50 mg/well a sharp decline in germination was exhibited.

At 90 DAS, shoot powder from Wad Ahmed at10mg/well induced poor germination (34%). Increasing powder levels to 20 mg/well increased germination to71%. An increase in amount of shoot powder to 30 mg/well reduced germination significantly. A further increase in powder level to 40 and 50 mg/well affected no further significant effect on germination. Tetron shoot powder at10 and 20mg/well induced low germination (23 and 18%, respectively).Increasing powder amount to30mg/well increased germination significantly (Fig4.7.c).A further increase in powder level to 40 and 50 mg/well resulted in a significant decline in germination which was at par with the lower powder levels (10 and 20mg/well).Shoot powder from Hakika at 10mg/well induced poor germination (17%) (Fig4.7.c, Appendix .5). Increasing amount of powder to 20-40 mg/well increased germination, albeit not significantly. A further increase shoot powder to50 mg/well decreased germination, albeit not significantly (Fig4.7.c).

At 120 DAS, germination irrespective of sorghum genotype or amount of powder was poor (<40 %) (Fig4.7.d, Appendix .5). Shoot powder from Wad-Ahmed at 10 mg/well induced negligible germination (9 %).Increasing powder amount to 20 mg/well increased germination

significantly. Further increase of shoot powder to 30-50 mg/well, with single exception, decreased germination albeit not significantly (Fig4.7.d). Shoot powder from Tetron showed a comparable trend to that of Wad Ahmed. At 10 and 20 mg/well Tetron powder induced negligible germination (5-7%). Further increase in amount of powder to 30 mg/well increased germination to 24 %. A further increased in amount of powder to 40 and 50 mg/well showed inconsistent and negligible germination (<24%). For Hakika shoot powder induced negligible germination (3-14%) and differences between treatments were not significant (Fig4.7.d, Appendix .5).

Irrespective of powder amount mean germination inducing activity was maximal for powder collected at 40 DAS followed in descending order by powder collected at 20, 90 and 120 DAS. At 20 DAS powder from Tetron displayed the highest mean germination inducing activity (48.7%) followed in descending order by Wad Ahmed (43.8%) and Hakika (28.8%). At 40 DAS, Wad Ahmed scored the highest mean germination (51.7%) followed by Tetron (44.8%) and Hakika (44.1%). However, differences were not significant. At 90 DAS, Wad Ahmed shoot powder displayed the highest mean germination (43.7 %), followed by Tetron (32.7 %) and Hakika (22.1 %) with significant differences. At 120 DAS, through germination inducing activity was negligible and the lowest, shoot powder from Wad Ahmed displayed the highest germination inducing activity (17.1%), followed by Tetron (10.2 %) and Hakika (6.8 %).

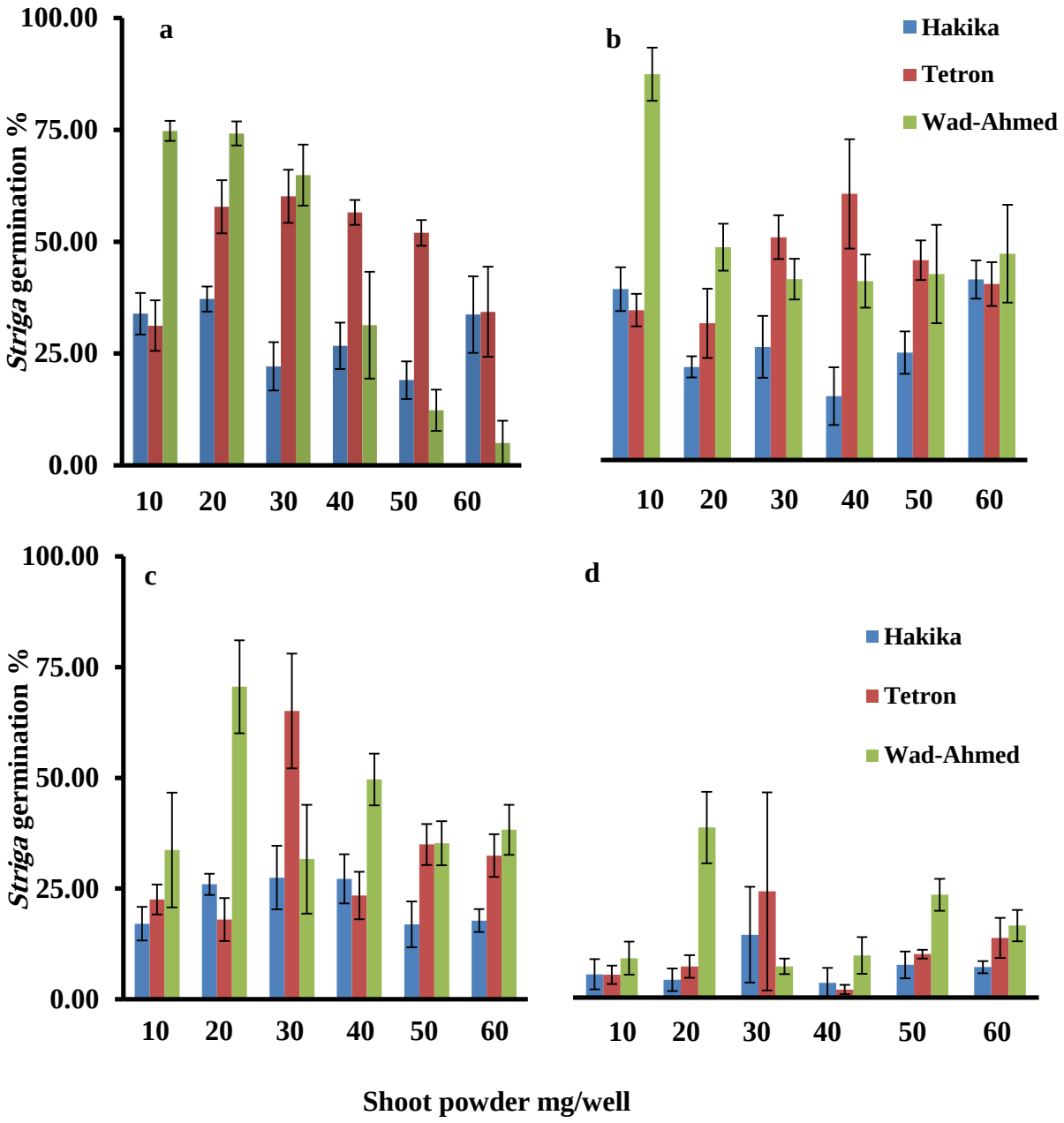

Fig.4.7. Effects of genotype and growth stage on germination inducing activity of sorghum shoot powder a) 20 DAS, b) 40DAS, c) 90 DAS and d) 120 DAS. Vertical represented stander error.

4.3.1.1. 2. Germination inducing activity of root powder: -

In general germination inducing activity of root powder progressively increased with increasing powder amount, reached a maximum and subsequently declined (Fig 4.8, Appendix .6).

At 20 DAS, Wad Ahmed root powder at 10 mg/well induced 68% germination increasing powder to 20 mg/well increased germination to 93%. On further increase in root powder level to 30 mg/well germination declined to 84%.Increasing powder amount to 40 and 50 mg/well decreased germination to 73.8 and 73.9 %, respectively. (Fig 4.8.A, Appendix .6). Tetron root powder at 10 mg/well induced 66% germination (Fig 4.8.a, Appendix .6).Increasing powder amount to 20 and 30 mg/well increased germination to 85, 88% respectively. A further, increase in amount of root powder to 40 and 50 mg/well decreased germination significantly (60, and 54 % respectively). Hakika root powder at 10 mg/well induced 27% germination (Fig4.8.a, Appendix.6). Increasing powder amount to 20 mg/well, increased germination to 37%. Increasing amount of powder to 30, 40 and 50 mg/well progressively decreased germination. However, the decrease in germination was significant, only, at the highest powder amount (Fig 4.8.a, Appendix .6).

At 40 DAS, powder from Wad Ahmed at 10 mg/well induced 68% germination. Increaseing amount of powder to 20 and 30 mg/well induced the highest germination (82%) (Fig 4.8.b, Appendix .6). A further, increase in amount of powder to 40 and 50 mg/well resulted in non-significant decline in germination (62 and 77%, respectively) (Fig 4.8.b, Appendix .6). Tetron root powder at10 and 20 mg/well induced 63 and 68% germination, respectively.Increasing amount of powder to 30-50 mg/well decreased germination to 50–60 % with no significant differences. Hakika root powder at 10mg/well induced poor germination (14%). Increasing root

powder to 20 mg/well increased germination significantly to 48 %. A further, increase in amount of powder to 30 -50 mg/well showed inconsistent induction of germination (35-54%) (Fig4.8.b, Appendix .6).

At 90 DAS, root powder from Wad Ahmed, irrespective of amount, induced high germination (73-91%) and with a single exception, differences in the elicited germination were not significant (Fig 4.8.c, Appendix .6). Germination induced by powder from Tetron at 10 mg/well was 76%.Increasing powder amount to 20-40 mg/well increased germination to 83-96%, with no significant difference between treatments.Increasing powder levels to 50 mg/well decreased germination significantly (Fig 4.8.c, Appendix .6). Hakika root powder at 10 mg/well induced (52%) germination (Fig 4.8.c, Appendix .6). Increasing levels to 20 and 30 mg/well increased germination significantly (>80%). A further increase powder amount to 40 mg/well or more resulted in significant decline in germination.

At 120 DAS, negligible germination (<10%) was achieved irrespective of genotype or powder amount. Among the genotypes mean germination was 6, 1.5 and 1.2 for powder from Wad Ahmed, Tetron and Hakika, respectively (Fig 4.8.d, Appendix .6). For amount of powder, irrespective of genotype, mean germination varied with growth stage and was maximal (87.2 %) at 90 DAS and minimal (1.2%) at 120 DAS. Irrespective of powder amount and growth stage mean germination was invariably maximal for Wad Ahmed (96.6 – 93.3 and 92.7 %) and minimal for Hakika (13.8 –9.0 and 0.0 %).

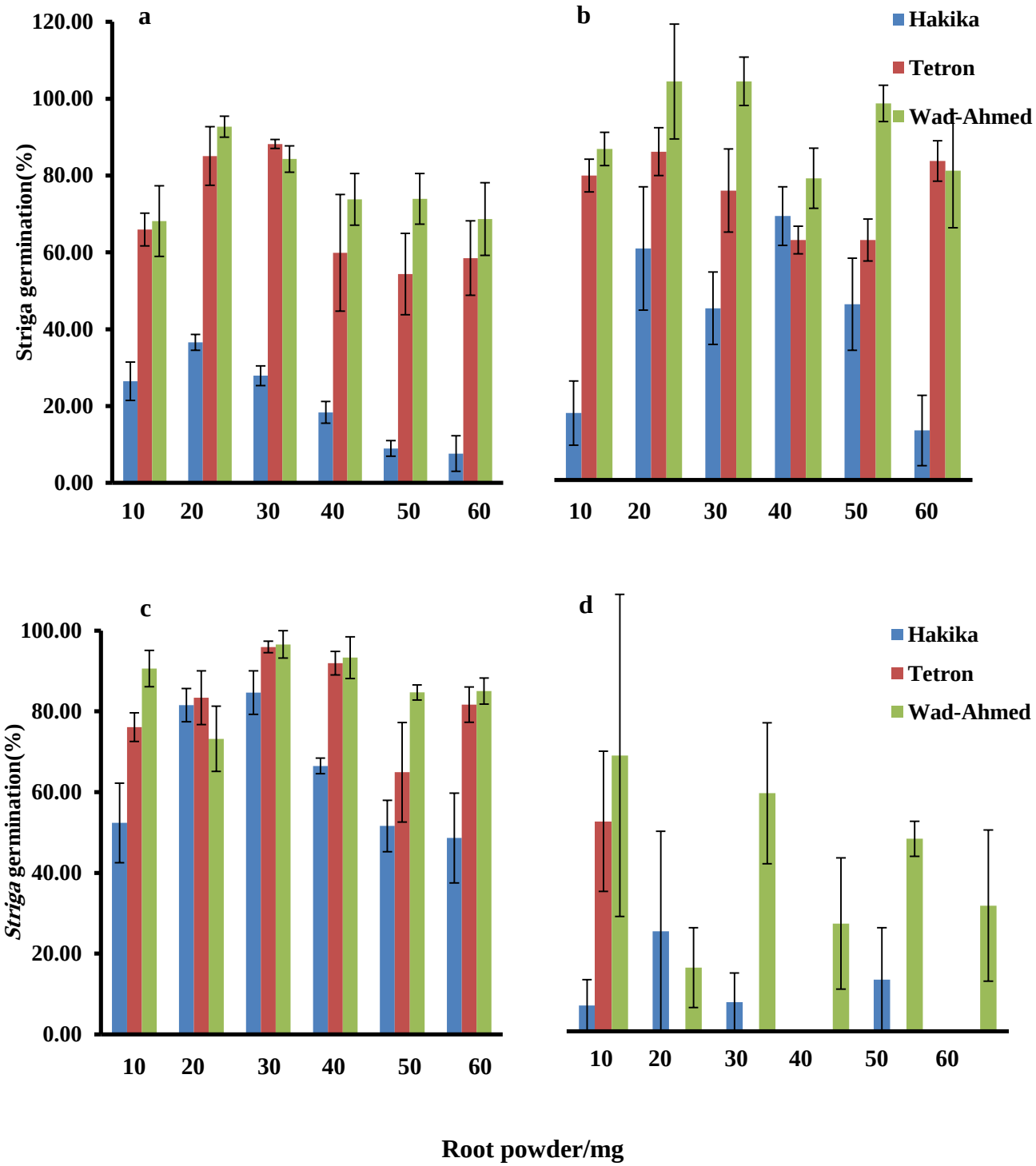

Fig.4.8. Effects of genotype and growth stage on germination inducing activity of sorghum root powder a) 20 DAS, b) 40 DAS, c) 90 DAS and d) 120 DAS. Vertical bars represent stander error.

4.3.1.3. Effects on Radicle length: -

4.3.1.3.1. Effect of shoot powder on radicle length:-

Striga seedlings from seeds induced to germinate by sorghum shoot powder, irrespective of genotype,powder level or sampling date displayed radicle length of 0.1- 0.6 µm $\times 10^{-2}$ (Fig 4.9, Appendix .7).

At 20 DAS, shoot powder from Wad Ahmed at 10 and 20 mg/well induced radicle length of 0.18-0.2 µm$\times 10^{-2}$.However, increasing amount of powder to30 mg/well or more reduced radicle length, albeit not significantly (Fig 4.9.a, Appendix .7). Seedlings from seeds induced to germinate by Tetron shoot powder at 10 mg/well, displayed radicle length of 0.98 µm$\times 10^{-2}$. A further increase in powder amount to 20-40 mg/well reduced radicle length, albeit not significantly. However, a further increase in powder amount to 50 mg/well affected a significant reduction. Hakika shoot powder at 10 and 20 mg/well sustained radicle length of 0.4 and 0.43 µm$\times 10^{-2}$,respectively (Fig 4.9.a, Appendix .7). Increasing powder levels to 30 mg/well or more reduced radicle length. The reduction in radicle length was highest, albeit not significant at 50 mg/well.

Radicle length, irrespective of genotype, was maximal (0.52µm$\times 10^{-2}$) at 10mg/well and minimal (0.2 µm$\times 10^{-2}$) at 50 mg/well. Among genotypes shoot powder from Tetron showed, significantly, the tallest radicles, whereas powders from Wad Ahmed and Hakika sustained significantly shorter radicles (Fig 4.9.a, Appendix .7).

At 40 DAS Wad Ahmed shoot powders at 10 mg/well sustained a radicle length of 0.28 µm$\times 10^{-2}$.Increasing powder amount to 20 mg/well or more reduced radicle length, albeit not significantly (Fig 4.9.b, Appendix .7). Tetron shoot powder at 10 mg/well sustained radicle length of 0.28µm$\times 10^{-2}$, increasing powder amount to 20 mg/well or more had no significant effect on radicle length. Hakika shoot powder at 10 mg/well sustained a radicle length of 0.33µm$\times 10^{-2}$.Increasing powder amount to 20 mg/well

increased radicle length slightly. A further increase of shoot powder to 30 mg/well resulted in inconsistent and non-significant reductions. Radicle length, irrespective of sorghum genotype, was maximal at 10 mg/well powder level. Increasing powder amount above 10 mg/well reduced radicle length, albeit not significantly (Fig 4.9.b, Appendix .7). Irrespective of amount, shoot powder from the three genotypes sustained comparable radicle length (Fig 4.9.b, Appendix .7).Hakika shoot powder at 10 mg/well sustained radicle length of 0.3µm×10^{-2}. Increasing powder amount to 20, 30 and 40 mg/well reduced radicle length, albeit not significantly. However, at a powder level of 50 mg/well a significant decrease in radicle length was realized. Radicle length, irrespective of genotype, was maximal at 10 mg/well and minimal at 50 mg/well. Irrespective of powder amount all genotypes sustained comparable radicles (Fig 4.9.b, Appendix .7).

At 90 DAS, Wad Ahmed shoot powders at 10 mg/well sustained radicle length of 0.20 µm×10^{-2}.Increasing powder level to 20 mg/well or more had no effect on radicle length. Tetron shoot powder at 10 mg/well sustained radicle length of 0.38 µm×10^{-2}. Though, radicle length decreased with increasing amount of shoot powder, but differences between treatments were not significant (Fig 4.9.c, Appendix .7).Hakika shoot powder at 10 mg/well sustained radicle length of 0.3µm×10^{-2}. Increasing powder amount to 20, 30 and 40 mg/well reduced radicle length, albeit not significantly. However, at powder level 50 mg/well a significant decrease in radicle length was realized. Radicle length irrespective of genotype was maximal at 10 mg/well and minimal at 50 mg/well. Irrespective of powder amount all genotypes sustained radicle of comparable length (Fig 4.9.c, Appendix .7).

At 120 DAS, powder from Wad-Ahmed sustained radicle length of 0.2 µm×10^{-2}. A further, increase of powder level to 20 mg/well or more displayed no significant effect on radicle length (Fig4.9.d, Appendix

.7).Powder from Tetron at 10 mg/well sustained radicle length of 0.15 µm×10^{-2}. Increasing powder level to 20 mg/well or more had no significant effect on radicle length. Hakika shoot powder at all levels sustained inconsistent radicle length (Fig 4.9. d, Appendix .7). Irrespective of genotype, radicle length was maximal at 40 mg/well and minimal at 30 mg/well. Among genotypes shoot powder from Tetron and Wad Ahmed showed the tallest radicle length, whereas that from Hakika sustained significantly lower radicle length (Fig 4.9.d, Appendix .7).

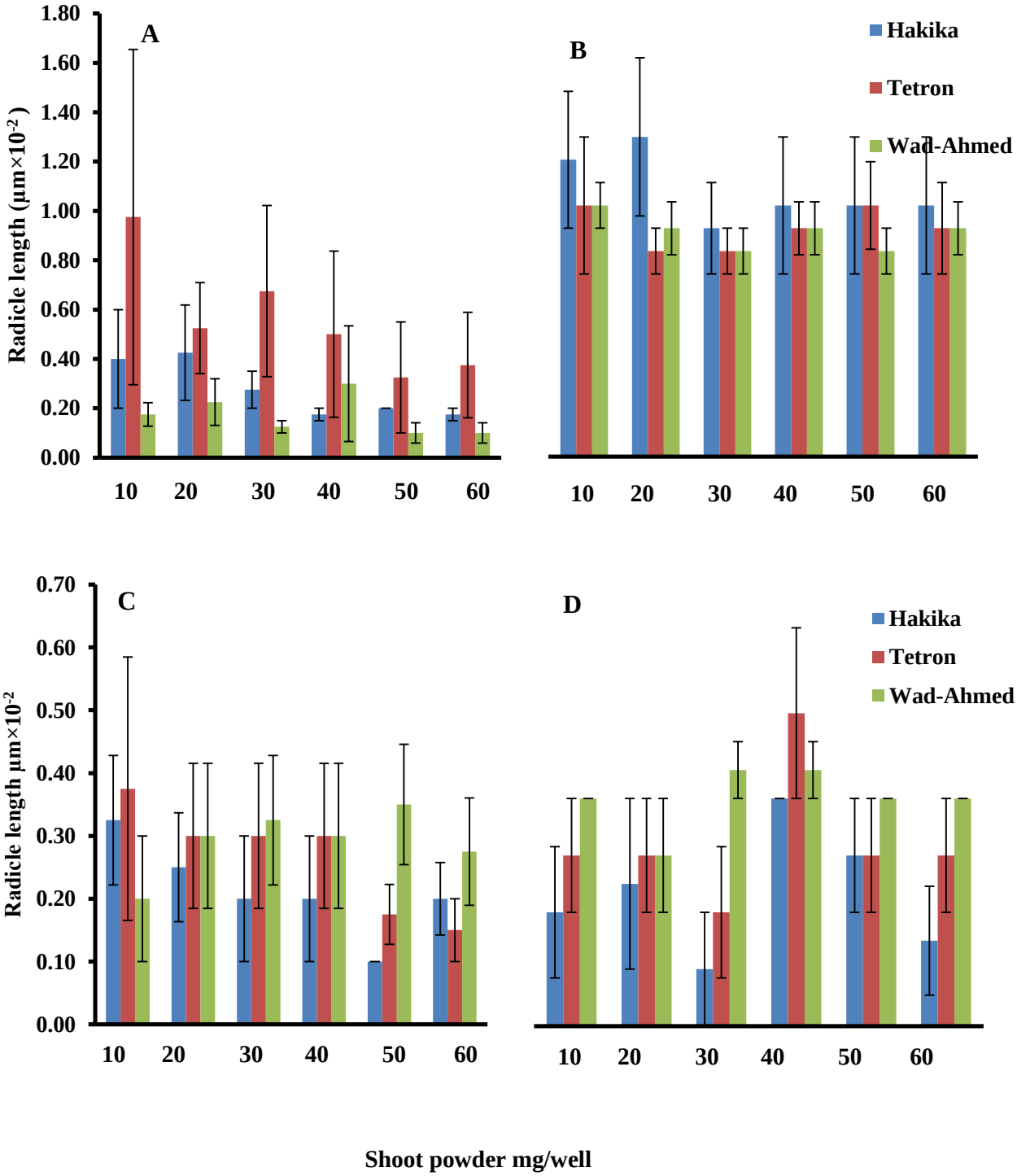

Fig4.9. Effects of genotypes, growth stage and amount of sorghum shoot powder on radicle length of *S. hermonthica* a) 20 DAS, b) 40DAS, c) 90 DAS and d) 120 DAS. Vertical bars represent stander error.

4.3.1.3.2. Effect of root powder on radicle length: -

At 20 DAS root powder from Wad Ahmed at 10 mg/well sustained radicle length of 0.3µm10^{-2}(Fig 4.10.a, Appendix .8).Increasing powder amount to 20 and 30 mg/well reduced radicle length significantly. A further increase in powder level to 40 mg/well or more resulted in a further significant decrease in radicle length.Tetron root powder at 10 mg/well sustained radicle length of 0.15× µm10^{-2} Increasing powder amount to 20 mg/well or more decreased radicle length significantly (Fig 4.10.A, Appendix .8). Hakika root powder sustained radicle length of 0.1µm10^{-2} in all treatments (Fig 4.10.a, Appendix .8). Irrespective of powder level, Wad Ahmed sustained significantly the tallest radicle length (0.22×µm10^{-2}) in comparison to its congeners Tetron and Hakika. Tetron and Hakika supported comparable radicle length. Irrespective of genotype, radicle length was significantly highest at 10 mg/well.

At 40 DAS Wad Ahmed root powder at 10 mg/well sustained radicle length of 0.25× µm10^{-2}(Fig 4.10.b, Appendix .8).Increasing powder amount to 20 mg/well or more decreased radicle length significantly. Tetron at 10 mg/well sustained radicle length of 0.15× µm10^{-2}.Increasing powder amount to 20 and 30 mg/well increased radicle length significantly. A further increase in powder amount to 40 and 50 mg/well reduced radicle length significantly. Hakika root powder, irrespective of amount, consistently sustained shorter and comparable radicle length (Fig 4.10.b, Appendix .8). Irrespective of genotype radicle length was maximal at 10 mg/well and minimal at 40 and 50 mg/well.

At 90 DAS, root powder from Wad Ahmed at 10 mg/well affected the tallest radicle length (1.15 µm×10^{-2}) (Fig 4.10.c, Appendix .8). Increasing powder amount to 20 and 30 mg/well reduced radicle length, albeit not significantly. A further increase in powder amount to 40 and 50 mg/well decreased radicle length significantly. Tetronroot powder at 10 mg/well

sustained radicle length of 0.9 µm×10^{-2}. Increasing amount of powder to 20 mg/well increased radicle length albeit not significantly. A further increase to 30 mg/well or more decreased radicle length significantly. Hakika root powder supported inconsistent radicle length with no significant differences between powder amounts (Fig 4.10.c, Appendix .8). Irrespective of powder amountWad-Ahmed and Tetron supported comparable mean radicle length whereas their congener from Hakika sustained significantly shorter radicle length. Irrespective of genotype, radicle length was maximal at powder amount of 10 - 30 mg/well and minimal at 40 and 50 mg/well.

At 120 DAS, irrespective of genotype or powder amount radicle length was very short (0.00- 0.1 µm×10^{-2}) with no significant differences across powder amount or across genotypes (Fig 4.10.d, Appendix .8).

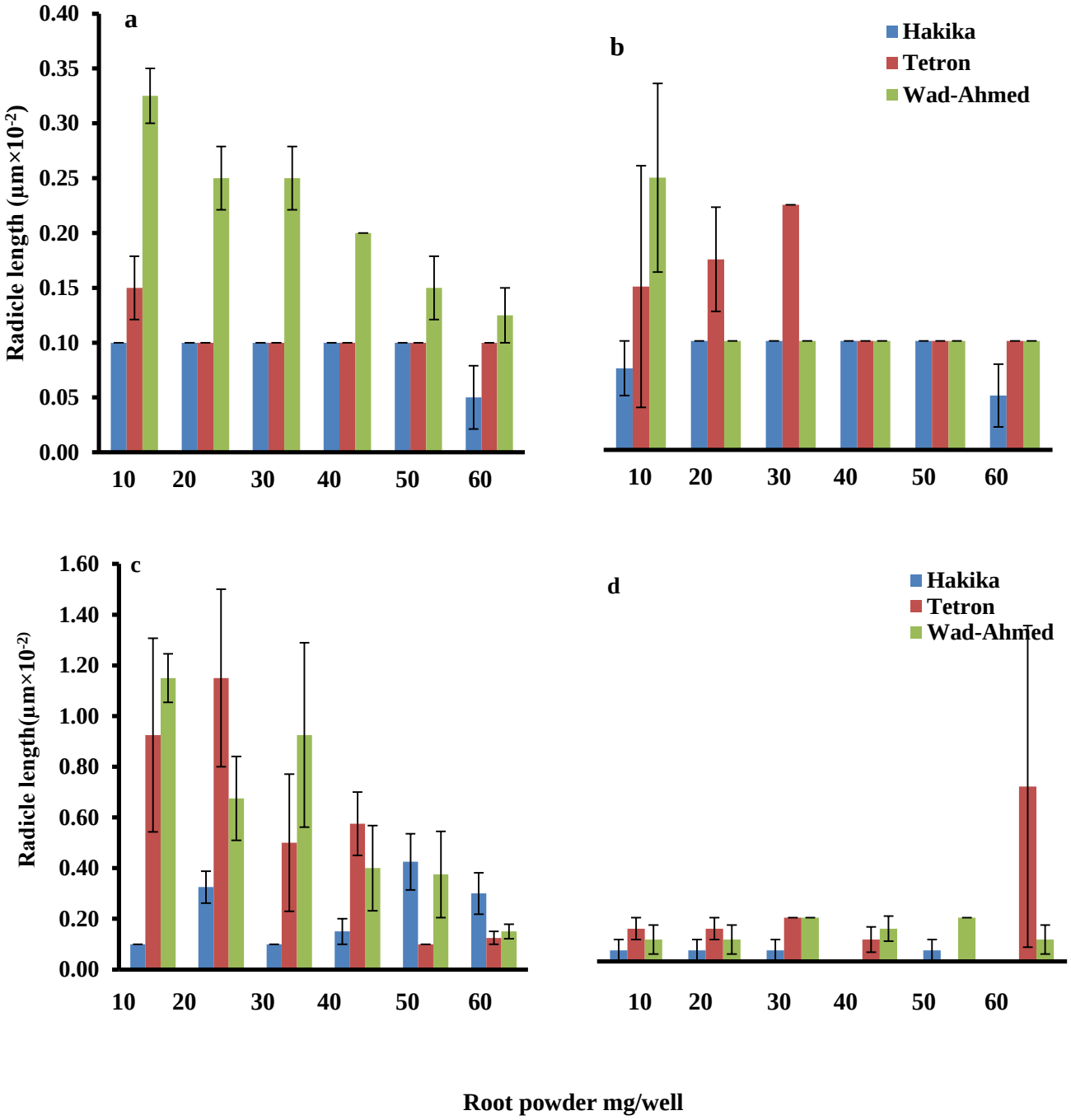

Fig.10. Effects of genotypes, growth stage and amount of sorghum root powder on radicle length of *S. hermonthica* a) 20 DAS, b) 40DAS, c) 90 DAS and d) 120 DAS. Vertical bars represent stander error.

4.3.2. Effects of sorghum root residues on germination, radicle elongation and haustorium initiation in *S. hermonthica*: -

4.3.2. 1.Effects on germination: -

Root residue assayed subsequent to harvest (DSH) for germination inducing activity showed differential performance (Table 4.8, Appendix .9). At 60 DSH, root residues from Wad Ahmed induced little to negligible germination (3.1-20.8 %) with no significant differences in activity. The maximum germination 20.8 % was attained at 30 mg. Germination inducing activity of roots residues from Tetron was low (3.9 -12% germination) at 10 and 20 mg/well. However, a significant increase in germination inducing activity occurred at 30 and 40 mg/well.A further increase in powder amount to 50 mg/well suppressed germination significantly (Table 4.8, Appendix.9). Germination inducing activity of roots residues of Hakika was low (0-17 %) with no significant differences between treatments. Across genotypes, root residues from Tetron displayed, significantly the highest mean germination inducing activity (24.7% germination). Root residues from Wad Ahmed and Hakika displayed the lowest mean germination (8.9 and 6.2%, respectively). Across residues levels germination inducing activity increased with increasing residues amount reaching significance (26.7% germination) at 30 mg /well. Increasing residue amount to 40 mg/well decreased germination inducing activity, albeit not significantly. A further increase in amount of residues to 50 mg reduced germination significantly to 9.3 (Table 4.8, Appendix .9).

At 75 DSH, root residues from Wad Ahmed and Tetron, displayed low and comparable germination inducing activity (19.2-32.9% germination) with no significant differences between residue levels (Table 4.8, Appendix .9). Root residues from Hakika, on the other hand, displayed significantly lower germination inducing activity with no significant differences across

residues amounts. Across residue amount germination was low (15.6-21.2% germination) with no significant differences (Table 4.8, Appendix .9).

At 90 DSH, root residues of Wad Ahmed displayed low to moderate germination inducing activity (30.4-50% germination) with no significant differences between residues amount. Tetron root residue showed variable germination inducing activity which increased with increasing amount of root residues reaching a maximum (38.2 % germination) at powder amount of 40 mg/well and subsequently declined on further increase of powder amount with no significant differences between treatments (Table4.8, Appendix.9). Root residues from Hakika displayed inconsistent germination inducing activity which varied between 4 and 40.9% with no significant differences between treatments.

Table .4.8. Effects of sorghum root residues on germination of *S. hermonthica*

Days after sowing (DAS)							
60 DAS							
Powder amount (mg/well)							
Cultivars	**10**	**20**	**30**	**40**	**50**	**60**	**Mean**
Wad- Ahmed	7.1 c	7.8 c	20.8 bc	5.7 c	3.1 c	8.7 c	**8.9 b**
Tetron	3.9 c	12.0 c	42.4 ab	60.0 a	22.0 bc	8.0 c	**24.7 a**
Hakika	3.1 c	0.0 c	17.0 c	5.0 c	2.9 c	8.9 c	**6.2 b**
Mean	**4.7 b**	**6.6 b**	**26.7 a**	**23.6 a**	**9.3 b**	**8.5 b**	
75 DAS							
Wad- Ahmed	24.5 ab	19.5 ab	16.2 ab	25.5 ab	22.7 ab	25.1 ab	**22.3 a**
Tetron	23.9 ab	19.2 ab	25.0 ab	26.9 ab	27.3 ab	32.9 a	**25.9 a**
Hakika	8.7 ab	8.1 ab	6.5 ab	2.1 b	7.4 ab	5.7 ab	**6.4 b**
Mean	**19.1 a**	**15.6 a**	**15.9 a**	**18.2 a**	**19.1 a**	**21.2 a**	
90 DAS							
Wad- Ahmed	34.6 a	34.8 a	50.0 a	33.4 a	49.6 a	30.4 a	**38.8 a**
Tetron	20.8 a	28.5 a	23.2 a	38.2 a	11.2 a	15.3 a	**22.9 b**
Hakika	27.3 a	30.9 a	24.2 a	4.2 a	31.0 a	40.9 a	**26.4 ab**
Mean	**27.6 a**	**31.4 a**	**32.5 a**	**25.3 a**	**30.6 a**	**28.8 a**	

Means within a row or a column followed by the same letter(s) are not significantly different according to LSD at 5%.

4.3.2. 2.Effect on radicle length: -

At 60 DAH, *Striga* seedlings from seeds induced to germinate by root residues of all genotypes displayed very short radicle length (0.02 µm $\times 10^{-2}$) which was not affected by changing residues amount (Fig.4.11.a, Appendix .10). Seedlings from seeds induced to germinate by root residues from Tetron displayed extremely short radicle length (0.01 µm $\times 10^{-2}$). Increasing residue amount to 20 mg/well increased radicle length to 0.02 µm $\times 10^{-2}$ which stayed constant up to a residue amount of 50 mg/well and subsequently declined to 0.01 µm $\times 10^{-2}$ on further increase of residue amount to 60 mg/well. Radicle length of seedlings from seeds induced to germinate by root residues of Hakika was inconsistent and varied between 0.0 and 0.05 µm $\times 10^{-2}$. A cross genotypes seedlings from seeds induced to germinates by root residues from Wad Ahmed and Tetron showed significantly longer mean radicle length (0.02 µm $\times 10^{-2}$) than their congeners from Hakika (0.01 µm $\times 10^{-2}$). Across residue amounts mean radicle length was maximum and significantly the highest at the lowest residue amount (10 mg/well) However, higher residue amounts affected inconsistent radicle length and differences between treatments were often not significant (Fig.4.11.a, Appendix .10).

At 75 DAS, seedlings from seeds induced to germinate by root residues from Wad Ahmed displayed short (0.03-0.06 µm $\times 10^{-2}$) radicle length. The radicle length was significantly the highest at the lowest residue level (10 mg/well). Increasing residue levels to 20 mg or more resulted in variable and inconsistent radicle length (0.03-0.04µm$\times 10^{-2}$) with insignificant differences between treatments Seedlings from seeds induced to germinate by root residues from Tetron displayed short and inconsistent radicle length (0.02 -0.03µm $\times 10^{-2}$) with no significant differences between treatments. Seedlings from seeds induced to germinate by root residues from Hakika displayed short radicle length (0.02-0.040µm$\times 10^{-2}$) with no significant

differences between treatments. Across residues amounts, irrespective of sorghum genotype, differences were not significant (Fig.4.11.b, Appendix .10). Among genotypes Wad Ahmed showed the longest mean radicle length (0. 04µm $\times 10^{-2}$). Root residues from Tetron and Hakika, on other hand affected the shortest mean radicle length (0.03 µm$\times 10^{-2}$). However, differences in mean radicle length were not significant (Fig.4.11.b, Appendix .10).

At 90 DSH, seedlings from seeds induced to germinate by root residues from Wad Ahmed or Tetron displayed short radicle length (0.03-1.0 µm $\times 10^{-2}$) with no significant differences between treatments. Seedlings from seeds induced to germinate by Hakika displayed inconsistent radicle length which varied between 0.03 and 0.31 µm $\times 10^{-2}$ and was maximal at 50 mg/well. Across genotypes seedlings from seeds induced to germinate by root residues from Hakika displayed significantly the longest mean radicle, while those from seed induced to germinate by residues from Wad Ahmed and Tetron showed significantly shorter mean radicle length (Fig.4.11.c, Appendix.10). Across residue levels differences in radicle length were inconsistent.

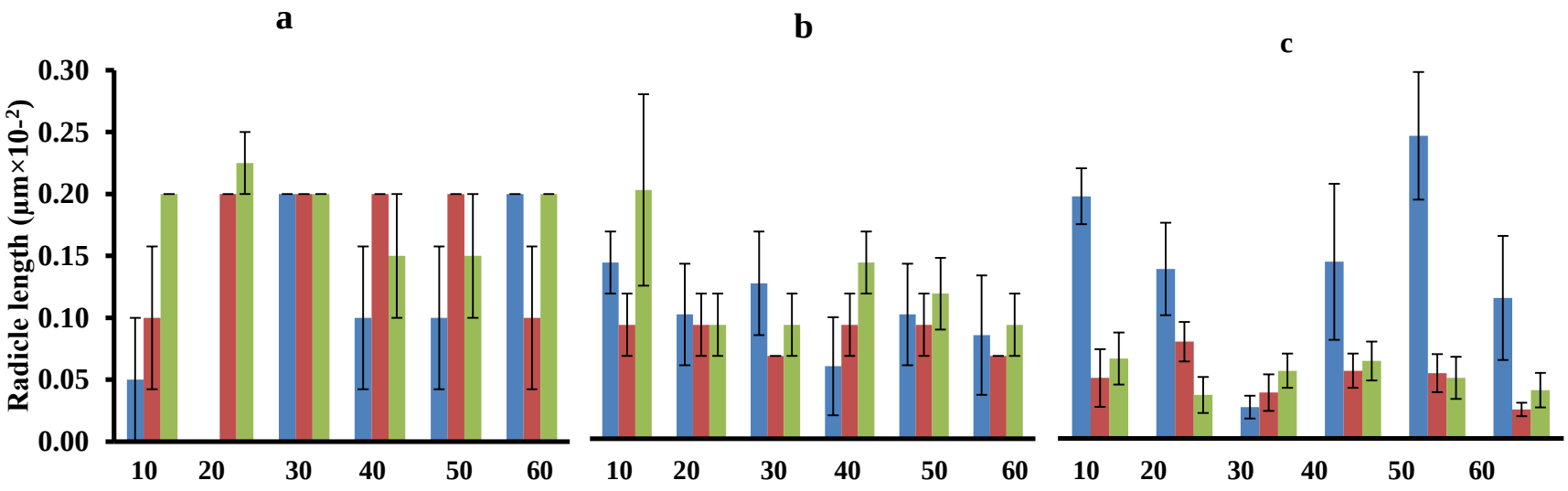

Fig.4.11. Effects of sorghum root residues on radicale length of *S. hermonthica* a) 60 DAS, b) 75DAS and c) 90 Vertical bars represent stander error.

4.4.3.2. 3.Effect on haustorial initiation: -

At 60 DSH, root residues assayed for haustorial initiation with time after harvest showed considerable differences in activity (Fig4.12.a, Appendix .11). Residues from Wad Ahmed induced high haustorial initiation (75-100%) with no significant differences between residue amounts. For root residues from Tetron haustorial initiation increased with residues, reached a peak (100 % haustorial initiation) at 30 mg per well with no further change up to 50 mg/well. Root residues from Hakika induced low, insignificant and inconsistent haustorial initiation (0 and 25%) at the lowest residue amounts (10 and 20 mg/well). Increasing residue amount to 30 mg/well or more, resulted in significant increase in haustorial induction (50-100%) (Fig4.12.a, Appendix .11). A cross genotypes root residues from Wad Ahmed and Tetron induced comparable haustorial initiation (92 and 77 %, respectively). Root residues from Hakika, on other hand, affected significantly lower haustorial initiation. A cross residue levels haustorium initiation was lowest (54 and 58%) at 10 and 20 mg/well. At 30 mg haustorium induction was maximal (92%). However, further increase in residue levels reduced haustorium formation, albeit not significantly (Fig4.12.a, Appendix .11).

At 75 DSH, root residues from Wad Ahmed, with a single exception, affected very high haustorial initiation (96-100 %). Root residues from Tetron affected 100 % haustorium initiation at the lowest level (10 mg). Increasing residue levels to 30 mg or more resulted in inconsistent reductions in haustorium initiation (Fig.4.12.b, Appendix .11). Among genotypes root residues from Wad Ahmed and Tetron affected comparable haustorial initiation (94-100 %, respectively). Root residues from Hakika, on other hand, affected the lowest haustorium initiation (63%). Across residue levels haustorium induction was maximal (100%) at the lowest

residue level (10 mg). Increasing residue levels reduced haustorium induction, albeit not significant (Fig4.12, b, Appendix .11).

At 90 DSH, root residues generally affected high haustorium initiation (Fig4.12.c, Appendix .11). In general, haustorium initiation by Root residues from Wad Ahmed progressively increased with residue amount up to 40mg/well and subsequently declined, albeit non-significantly. Root residues from Tetron, induced high haustorium initiation (87.3-100%) with no significant differences between residue amounts. Root residues from Hakika affected moderate to high haustorium initiation and with single exception differences in activity between residue amounts were not significant. Among genotypes haustorium induction was highest (93%) for Tetron and lowest (82%) for Hakika; however, differences were not significant. A cross residue levels haustorium induction was maximal (93%) at 40 mg and lowest (80%) at 30 mg. However, differences in activity between residue amounts were not significant (Fig4.12.c, Appendix .11).

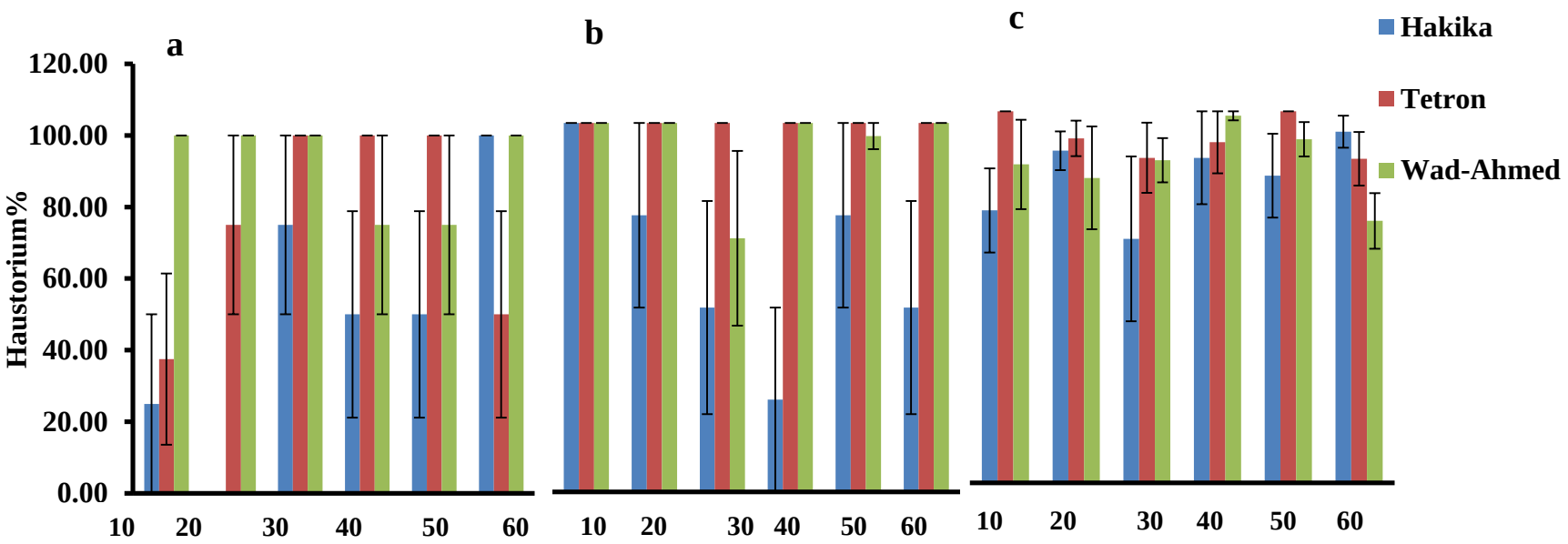

Fig.4.12. Effects of sorghum root residues on haustorial initiation *S. hermonthica* a) 60 DAS, b) 75DAS and c) 90.Vertical bars represent stander error.

CHAPTER FIVE

Discussion

Parasitic weeds are a menace that causes high yield losses in crops of economic importance across the globe(Babiker *et al.*, 2007).Recent studies revealed the complex nature of the host/parasite relationship and how it is governed by a system closely interwoven with the biotic and abiotic components of the environment and that the part played by the host, albeit instrumental, can be overwhelmed by environmental variables a fact that necessitates an integrated management strategies to safe guard against the spatiotemporal variables including the parasite seed bank, soil fertility and climate variables (Dernnan and Elhewrith, 1979, Stewart and Press, 1990; Press and Graves, 1991, Kar, 2011).

Intensive research on parasitic weeds started since the turn of the twentieth century and many control methods, have been recommended with limited adoption. The low adoption rate is attributed to the high costs of the inputs which make them a mismatch to the low inputs production systems fostered by socioeconomic factors and risk aversion particularly in developing countries and marginal areas where most of these weeds flourish and where crops yields are generally low. Resistant/tolerant crops appeared to be the simplest and easiest solution. However, there is no complete immunity and resistance to parasitic weeds is defined as the ability of the host to with stand the parasite attack and prevent or curtail its establishment or growth. Tolerance, on the other hand, is to with stand damage inflicted by the parasite. Further, resistance is categorized as pre-attachment or post-attachment. Pre-attachment resistance is based on mechanisms that allow a potential host to avoid or prevent attachment. Of these mechanisms low germination inducing activity of root exudates is the most studied (Ejeta, 2007). Post-attachment resistance occurs once the haustorium attaches and

attempts to penetrate the host roots tissues and establish connection with the vasculature. During these developmental stages (penetration of the host roots, ingression through the cortex and endodermis, establishment of connection with the vascular system up to maturity and seeds setting) constitutive or induced incompatibility leads to resistance (Timko and Scholes, 2013).

Three sorghum genotypes, Wad Ahmed, designated as *Striga* tolerant and Tetron and Hakika, renowned for pre-attachment resistance associated with low stimulant production (Gobena *et al*., 2017.) were selected for this study encompassing laboratory, greenhouse and field studies with the primary objective of developing affordable means for management of *S. hermonthica* in sorghum.

The laboratory investigations showed that germination affected by roots exudates obtained from hydroponically grown plants and in situ germination using the rhizotron technique, although showed a trend consistent with what was reported on involvement of stimulants production in reactions of the three genotypes (Table4.1) suggests clearly and for the first time that low stimulant production is not the sole mechanism of resistance to the parasite in Hakika and Tetron.

Root exudates from hydroponically grown plants showed that exudates from Wad Ahmed induced the highest germination in comparison to Tetron and Hakika up to 21 DAS. However, at 28 DAS root exudates from Tetron displayed the highest germination inducing activity. The observed high germination inducing activity of Wad-Ahmed root exudates up to 21 DAS is consistent with reports based on high production of 5-deoxystrigol compared with Hakika and Tetron which produce mainly, orobanchol, a lesser active and a lesser stable SL (Gobena *et al*., 2017, Yoneyama, 2020). However, the high germination stimulation activity of root exudates of Hakika at 14 DAS and that ofTetron at 28 DAS is at variance with the

reported pre-attachment resistance, which is associated with low induction of germination. The observed discrepancy could be attributed to a plausible change in SLs profile with time. Screening for low stimulant production using the standard agar gel bioassay technique considers differential germination inducing activity at 72 h after incubation. However, the recent observation reported by Yonyama (2020) may offer a more plausible explanation to the discrepancy. Yoneyama (2020), reported that in contrast to the standard agar gel assay high and similar germination inducing activity of roots exudates from both Striga resistant, orobanchol producing sorghum genotypes and their susceptible 5-deoxystrigol producing congeners, were realized when the exudates were added directly to conditioned seeds. The observed contrasting performance is attributed to the short persistence of orobanchol in the agar gel bioassay and in soils. The short persistence of orobanchol, a hydroxylated SL, is attributed to rapid deactivation through ring destruction by neucleophlic attack (Gobena *et al*., 2016).

Striga germination stimulants, mainly strigolactones, are unstable in soil the ether-enol bond, in both canonical and non-canonical strigolactones makes them susceptible to nucleophelic substitution and thus abolish their germination inducing activity (Yoneyama, 2020). The instability of the stimulants plays a major role in controlling the distance between the host roots and the parasite where germination is permissible and is very important for survival of the parasite in view of its limited seeds energy reserves. Following germination the seedlings radicles, chemotactically attracted to the roots, directed by differential concentration of the stimulant on distal and proximal sides of the emerging radicle, come in close proximity of the roots for signal transduction and timely extraction of the haustorium inducing factor from the host roots.

A close examination of the data on response of radicle length to root exudates obtained from hydroponically grown plants revealed, irrespective of sorghum genotype, a decrease in radicle length with exudates volume. Further, irrespective of sorghum genotype or exudates volume mean radicle length increased with time and was maximal at 21 DAS. Further, mean radicle length affected by roots exudates of Hakika was invariably the highest while those from Wad Ahmed and Tetron showed fluctuations with time. Such patterns in variability in radicle length suggest changes in the profile of the root exudates and may reflect variability in amount of radicle elongation inhibitors and/or promoters with time. Further, variability in SLs profile and/or concentration affecting radicle length cannot be ruled out. Higher concentration of SLs were reported to reduce radicle length or even germination of Striga seeds (Parker and Riches, 1993).The plausibility of a role for SLs in roots exudates affecting radicle length, may be substantiated by the observed higher values of radicle length of Striga seedlings from seeds induced to germinate by root exudates from Hakika which is renowned for being a low Striga germination stimulants producer

Haustorium formation in response to root exudates varied with time and sorghum genotype. At 7 DAS haustorium formation was inconsistent and negligible.Across genotypes and exudates volumes only 0.0- 4.04 and 0.0-7.08% of the seedlings respectively, from distinct haustoria. The inconsistency between mean haustorium formation and root volumes for individual genotype or across root volumes is indicated by the low and insignificant correlations ($r = - 0.12$ and 0.10, respectively, $P \leq 0.01$). At 14 DAS However, haustorium formation, albeit still inconsistent, was relatively high for Wad Ahmed and Tetron (11.9 -33.6% and 12.2-31.8%, respectively), but significantly low for Hakika (0.0-8.7%). At 21 DAS haustorium formation was low and negligible for Tetron (0-8.3%) and Hakika (0.0-2.7%), with no apparent association with root volume ($r = -$

0.23 and - 0.26, P≤ 0.01). However, for Wad Ahmed haustorium formation was relatively high (16.9-43.4%) and displayed relatively a consistent pattern with exudates volume (r =0.50, P≤ 0.01). At 28 DAS haustorium formation was very low for Hakika (0.0-2.94%, r = - 0.31, P≤ 0.01) and Tetron (0.0-18.07%, r = - 0.70, P≤ 0.01), but was extremely high and consistent for Wad Ahmed as over 90%of the seedlings form haustoria(r =0.06, P≤ 0.01). The notable low haustorium formation in response to root exudates of Hakika and to some extent to that ofTetron is consistent with the notion that in sorghum the haustorium factor is not exuded by the host root, but it is a degradation product of the host lignin(Cui S *et al* ., 2016).

The only haustorium-inducing compound isolated from host roots is 2,6-dimethoxy benzoquinone (DMBQ) (Bandaranayake and Yoder,2013). Benzoquinones are ubiquitous in planta and are synthesized through the shikimate pathway, by oxidative decarboxylation of phenolic acids and by the enzymatic degradation of polyphenols by peroxidases and laccases. DMBQ was isolated from sorghum roots only after they were physically abraded or co-incubated with Striga cultures, processes that lead to the release of DMBQ through peroxidase-mediated oxidation of sorghum cellular components (Bandaranayake and Yoder, 2013). Hydrogen peroxide generated in Striga radicles is the rate limiting substrate for host peroxidases that catalyze the conversion of cell wall components into haustoria-inducing benzoquinones (Keyes *et al*., 2000). In this model, Striga radicles enzymatically extract DMBQ from the surface of host roots. However, the prolific haustorium formation observed for roots exudates of Wad Ahmed is at variance with the reported generation of DMBQ and suggests involvement of other signals in induction of haustoria. The observed anomaly in performance of Wad Ahmed root exudates is in line with the recent findings that several compounds isolated from rhizosphere of several plants *viz*cytokinins, which are structurally different from

phenoloic compounds, also trigger haustorium formation in Orobanchaceae (Goyet *et al*., 2017, Goyet *et al*., 2019).

The contribution of stability of SLs to the contrasting performance observed between the two assays protocols and its practical significance in screening for pre-attachment resistance is revealed further by the finding that the extremely instable recently discovered non-canonical SLs stimulate germination of parasitic weeds. Growing plants hydroponically and applying the resulting exudates directly to conditioned seeds, albeit may reflect the total activity of the blends, may lead to erroneous results.

Data from the laboratory experiment using the rhizotron technique as an assay method showed clearly that *Striga* germination progressively increased with time over the 3 weeks period. Further, in conformity with germination data from roots exudates of hydroponically grown plants insitu germination was invariably maximal for Wad Ahmed, intermediate for Tetron and minimal for Hakika. However, the differences in germination inducing activity between the genotypes were often not significant and may not account fully for the reported resistance in Hakika and Tetron. The notable staggered and/or progressive germination indicates that not all seeds were at the same physiological status and may thus be a survival mechanism as reported for seeds displaying physiological dormancy which possess several embryonic blocks inhibiting radicle protrusion (Baskin and Baskin, 2014; Brun, *et al*., 2018). *Striga* germination is a complex phenomenon. Germination stimulants, natural or synthetic, induce germination through elicitation of ethylene biosynthesis and ethylene triggers a series of biochemical reactions that influence abscisic acid/gibberellins (ABA/GA) balance in seeds leading to release of embryo dormancy and subsequently to germination (Babiker *et al*., 2000). The high germination inducing activity of root exudates of Wad Ahmed is consistent with the reported high contents of 5-deoxystrigol in the strigolactones

blend in its roots exudates (Nasreldin *et al*., 2016).*S. hermonthica* seeds, sorghum strain, are renowned for their high response to 5-deoxystrigol which is a strigol type strigolactone and for their low response to the orobancol-type strigolactones (Nasreldin *et al*., 2018; Gobena *et al.*, 2017). The relatively high germination inducing activity of roots exudates of the genotype Hakika and Tetron, though in contrast with previous reports (Nasreldin *et al*., 2016; Gobena *et al.*, 2017) is in line with the results obtained from root exudates of hydroponically grown plants and is congruent with the reported effects of assay medium on germination inducing activity of root exudates and relative instability of hydroxylated SLs and their non-canonical congeners as pointed out by Yoneyama (2020).

The rhizotron experiments further provides concrete evidence that low stimulant production is not the sole mechanism for resistance to Striga in Tetron and Hakika. Further, it showed clearly the involvement of post-attachment resistance at an advance stage of parasitism. Comparison of attachment expressed as percentage of the total number of germinated seeds for each of the genotypes across time showed little to negligible differences between genotypes and this is consistent with the notion that haustorium initiation and attachment are not specific as haustoria could attach to inert soil constituents (Riopel and Timko, 1995). The haustorium initiation factor in sorghum (2,6-DMBQ) is a lignin degradation product which results from activation of peroxidases in sorghum roots in response to a chemical signal (H_2O_2) released from the tip of the parasite radicle in close proximity of the host roots (Wada *et al*., 2019). However, based on number of attached seedlings the sorghum cultivar Wad Ahmed displayed the highest attachment followed in descending order by Tetron and Hakika. Further, irrespective of genotype attachment progressively increased with time and was maximal at 3 weeks.

The abrupt developmental arrest of the parasite at stage 2, which is an advance stage of parasitism as indicated by formation of two leaf pairs which indicate that the xylem-to-xylem connection had already been established (Hood *et al*., 1998), suggests, as previously reported for Framida (Arnaud, 1999), involvement of physiological mechanisms which impair nutrients and carbon acquisition by the parasite. This notion is substantiated by the notable death of the parasite seedlings attached to Hakika and Tetron. *Striga* seedlings mortality was maximal and highly significant on Tetron. However, on Hakika seedlings mortality albeit high, was at par with that on Wad Ahmed. Curtailment of nutrients transfer from the host to the parasite could be attributed to low haustorial competence and/or a physiological process that increases water and nutrient retention in host root and shoot tissues. *S. hermonthica* is reported to perturb the hormonal balance of its host. The parasite stimulates ABA accumulation and decreases that of GAs in its host (Taylor *et al*., 1996; Westwood, 2013). An increase in ABA concentration leads to a decrease in stomatal aperture, stomatal conductance, photosynthesis, host growth and increase the flow of the xylem sap from the host to the parasite (Taylor *et al*., 1996; Westwood, 2013). Equally well a decrease in GAs leads to stunted growth of the host and lessen it competitive ability (Taylor *et al*., 1996). Accordingly, it is plausible that differences in magnitude of the perturbation of the hormonal balance and/or differential sensitivity to the attained changes in hormonal balance between genotypes may alter the xylem flow from the host to the parasite. However, the possibility of vascular occlusion as noted for some sorghum genotypes (Timko and schools, 2013) cannot be refuted. In nature juvenile *S. hermonthica* plants at 6-8 weeks post-infection are subterranean and are totally dependent on their hosts for nutrients, water and carbon compounds essential for growth and survival. The results attained in this study is consistent with a model in

which the sorghum genotypes Tetron and Hakika resistance to *S. hermonthica* could be accounted for, at least in part, by physiological reactions which impair transfer of water, nutrients and carbon compounds from the host to the parasite.

Results from the greenhouse experiments showed that *Striga* emergence was invariably highest on Wad Ahmed, low on Tetron and lowest on Hakika and that emergence increased with seed bank size and time. Further, the increase in emergence with time is more consistent on Wad Ahmed, but was less and least consistent on Tetron and Hakika, respectively. *Striga* dry weight mirror imaged emergence of the parasite.

Variation in *Striga* emergence with genotype is in conformity with the reported resistance of Hakika and Tetron and tolerance in Wad Ahmed and is in line with the definition of resistance and tolerance as set by Rodenburg and Batiaans (2011). The results further indicate that Hakika, by virtue of sustenance of the lowest Striga emergence is more resistant than Tetron.Thus low germination and subsequently less infection pressure together with high Striga seedlings mortality, as noted in this study, could account, at least in part, for the low emergence of the parasite on Tetron and Hakika.

The differential response of sorghum growth attributes and traits as manifested in plant height, RLCC and dry weight to *Striga* seed bank size varied with genotypes and time. Early in the season (60 DAS) Wad Ahmed and Tetron showed a progressive, albeit non-significant, reductions in height reaching a maximum at the highest *Striga* seed bank size. For Hakika plant height was invariably comparable to that of the respective *Striga*-free control. At mid-season (90 DAS) Wad Ahmed displayed consistent reductions in height which reached significance at a seed bank of 4 mg/pot, Tetron, on the other hand, showed a consistent decline in height which reached significance at a seed bank of 6 mg/pot or more, whereas

Hakika, exhibited significance increase in plant height at a seed bank of 4 mg/pot or more. Rates of reduction in sorghum height were high and comparable for Wad Ahmed and Tetron, however, for Hakika plant height exceeded that of the *Striga*-free control.

At 60 DAS reductions in RLCC in Wad Ahmed were significant at seed bank size of 6 mg/pot or more. At 90 DAS significant reductions in RLCC were inflicted at a seed bank of 4 mg/pot or more. For Tetron and Hakika no significant, reductions in RLCC were realized at 60 DAS. At 90 DAS Tetron showed inconsistent reductions in RLCC which varied in significance with no clear association with seed bank size. For Hakika reductions in RLCC, with a single exception, were not significant. Likewise, reduction rates were high for Wad Ahmed and negligible and insignificant for Tetron and Hakika.

The three genotypes, with a single exception displayed considerable reductions in dry weight which varied in significance and association with seed bank size. For Wad Ahmed significant reductions (50.8-89.5%) were attained at all seed bank sizes. For Tetron reductions in dry weight were not significant at seed banks below 8 mg/pot. For Hakika reductions in dry weight (45.5-83.1%) were achieved at a seed bank of 4 mg/pot or more. It thus clear that Hakika, though more resistant than Tetron is less tolerant. This is consistent with expectation as Tetron is a local genotype, while Hakika is an exotic genotype. Further, the finding substantiates the report by Press and Stewart, (1987) that infection per se is more important than the degree of infection in determining the deleterious effects of *S. hermonthica*on host growth and physiology.

It is thus evident that the measured attributes and traits of the three sorghum genotypes were responsive to *Striga* infection. However, the magnitude and consistency of the response varied with the genotype and the attribute and/or the trait in question and may be as reported by Gurney

et al., (1999) due to the time frame of imposition of the infection. Wad Ahmed, where *Striga* emergence was the highest and most rapid consistently, displayed the highest reductions and reductions rates in height, RLCC and dry weight whereas Tetron and Hakika, where *Striga* emergence was low and late were less affected particularly at low *Striga* seed bank size

In general the stress instigated by *Stri*ga infection, as reported for many biotic and a biotic stresses (Tausz, *et al*,. 2004, Kar, 2011), may evoke a time series of responses in metabolic function of plants the outcome of which depends on the intensity of the stress, time of imposition and ability of the plant to acclimate to the stress factors and achieve a new steady-state. *Striga* is renowned for its ability to perturb the hormonal balance of its hosts. The parasite, in common with other biotic and a biotic stress is reported to increase ABA and decrease GAs in infected sorghum plants (Dernnan and Elhewrith, 1979, Stewart and Press, 1990; Press and Graves, 1995, Kar, 2011). An increase in ABA and a decrease in GAs together with the sensitivity to the change could account for the observed stunting in Wad Ahmed and Tetron as previously reported for *Striga* infected sorghum plants (Cardoso, *et al*., 2011). For Hakika, despite the increase in height, high losses in shoot dry weight were displayed thus indicating thinner stalks. However, the plausibility of involvement of more subtle interactions including complex physiological and biochemical interactions cannot be ruled out. Increased ABA level in plants is reported to induce stomatal closure, reduce photosynthesis, create an imbalance between the consumption of the reductant NADPH in assimilation and the need of the electron transport chain for the regenerated electron acceptor at the PSI site (NADP), lead to generation of reactive oxygen species (ROS) and thus increase the oxidative load in plants (Tausz, *et al*,. 2004, Kar, 2011). Overproduction and accumulation of ROS, as proposed for several biotic

and a biotic stress, may lead to cellular damage and impairs plants growth and development (Tausz, *et al*., 2004, Kar, 2011). Differential deactivation of ROS through antioxidant, enzymatic and/or non- enzymatic systems may account for the differential response noted between genotypes. It is noteworthy that in planta ROS, depending on concentration, could be protective or destructive and is linked with systemic acquired resistance (SAR) and systemic acquired acclimation (SAA) (Farooq *et al*., 2019).

Data from field grown plants, in congruence with those of the root exudates (Table 4.2), revealed dependence of germination inducing activity of sorghum shoot powder on genotype, amount of powder and growth stage. The three genotypes revealed three contrasting patterns of germination inducing activity. Germination inducing activity of shoot powder from Wad Ahmed collected early in the season (20 and 40 DAS) was highest at the lowest powder amount and decreased on increasing powder amount beyond a threshold level. Conversely the germination inducing activity of powder collected late in the season (90 and 120 DAS) was low at low powder amount, but progressively increased with powder amount and subsequently declined. For Tetron germination inducing activity of shoot powder, irrespective of collection time, was low at low powder amount, displayed a sharp increase with powder amount and subsequently declined on further increase in powder amount, albeit not significantly. For Hakika germination inducing activity displayed insignificant initial increase with powder amount followed by insignificant decrease with increasing powder amount. It is noteworthy that germination inducing activity of powder from all genotype decreased with time and was often significantly the highest for Wad- Ahmed and lowest for Hakika. The notable initial and temporal variability in germination inducing activity between and within genotypes may be attributed to variations in stimulants and/or inhibitors amounts and/or profiles.

The decrease in germination inducing activity with time, as is expected, is consistent with the observed decline in root powder germination inducing activity and may reflect a decrease in synthesis and/or translocation of SLs late in the season. SLs are synthesized, mainly, in the roots and subsequently translocated acropetally to the shoot (Jamil *et al*, 2010, Kohlen, *et al*., 2010).

In general germination inducing activity of powder from roots of field grown plants was highest for Wad Ahmed, intermediate for Tetron and lowest for Hakika, These findings are consistent with previous reports that the stimulants blend in the root exudates of Wad Ahmed is predominated by 5-deoxystrigol which is the most active and relatively stable SL, while the root exudates of Hakika and Tetron are dominated by the less active and less stable SL (orobanchol) (Gobena *et al.,* 2017). However, as previously mentioned the unexpected higher germination inducing activity of Hakika and Tetron could be attributed to existence of the hydroxylated canonical (orobanchol) and its non-canonical congeners, which are relatively unstable and difficult to isolate (Yoneyama, *et al*., 2020). The observed decrease in the germination inducing activity with increasing root powder may be attributed to changes in stimulants and/or inhibitors profiles. The initial increase in germination inducing activity (20-90 DAS) and the observed decline at 120 DAS could be related to fluctuations in SLs biosynthesis with time. The SLs in addition to their effects on parasitic plants are hormonal in nature and they regulate plant growth and developments through cross-talk with other phytohormones, particularly ABA and auxins (Xie *et al.,* 2010; Al-Babili and Bouwmeester, 2015, Saeed, 2017).

In general radicle length, invariably, decreased with increasing shoot powder. In most cases radicle length was the tallest at the lowest powder amount and shortest at the highest powder amount, albeit differences were

often not significant. Furthermore, radicle length affected by powder collected at 20 DAS was significantly higher for Tetron. Powder from Hakika and Wad Ahmed affected seedlings with comparable radicle length. Powder collected 40 and 90 DAS, irrespective of sorghum genotype, affected seedlings with comparable radicle length. Seedlings affected by powder collected at 120 DAS resulted in seedlings with differential radicle length. Powder from Wad-Ahmed sustained the longest radicle length. Radicle length a ffected by powder from Tetron were shorter, albeit at par with those of seedlings from seeds induced to germinate by powder from Wad Ahmed. Powder from Hakika, on the other hand, affected the shortest radicle length. These finding, in line with previous results suggest involvement of inhibitory substance(s) which reduce cell extension and/or promote precocious haustorial initiation (Riopel and Timko, 1995; Khan *et al*., 2002, 2007). It deserves mentioning that radicle elongation in *Striga* and many allied parasites is attained through cell extension and not cell division (Brown, *et al*., 1949.Riopel and Timko, 1995; Khan *et al*., 2007).

The low and mostly inconsistent germination inducing activity of root residues indicates the considerable losses in germination inducing activity on exposure to field conditions after harvest. Both canonical and none-canonical SLs are renowned for short persistence. However, based on high proportion of haustoria (100- 94.2 and 93.2%) in the resulting seedlings precocious induction of haustoria may have had precluded radicle protrusion leading to the observed low germination.

Mean radicle length affected by root residues retrieved from field grown sorghum after harvest was very short (0.02-0.18µm). Further, radicle length was affected by genotype and time after harvest the residues were collected. At 60 DAH root residues from Hakika affected significantly the shortest radicles in comparison to its congeners from Wad-Ahmed and Tetron, whereas at 75 DAH root powder from the three genotypes affected

comparable radicle length. However, at 90 DAH radicle length sustained by powder from Hakika root was significantly the tallest. It is noteworthy that radicle length, albeit still short, increased with time lapse between harvest and sampling. The results seem to be an outcome of several interactions, involving the plant, the parasite and the environment. Striga radicle length is determined by extensibility of the cells. Perception of the haustorium factors results in halting radicle extension and according leads to radicle shortening and hence reduces contact between the host roots and the parasite (Riopel and Timko, 1995). However, the plausibility of involvement of toxic compounds diffusing from the residues and/or extracted by enzymes from the parasite or activated by reactive oxygen species diffusing form the Striga radicle cannot be ruled out. It has to be noted that peroxidases capable of oxidizing phenolic compounds to benzoquinone were isolated from radicles of seedlings of the closely related species *S. asiatica* (Kim *et al*., 1998).

The root residues of Wad Ahmed and Tetron, irrespective of powder amount, consistently induced high haustorium initiation. The genotype Hakika on the other hand showed inconsistent haustorium initiation which varied from negligible at lower powder amount to 60% at 75 DSH. However, a fairly high and consistent haustorium initiation, comparable to that of Wad Ahmed and Tetron, was achieved at 90 DSH. Based on radicle size, haustorium initiation occurred concurrently with germination and it could be a reason for the attained low radicle protrusion. Induction of precocious haustoria by root residues and/or weed residues needs to be further studyed as it could be developed into a novel control measure for root parasitic weeds as radicle extension is important to maximize contact between the parasite and its host. In nature subsequent to germination *Striga* radicle, chemotactically directed to the host roots, elongates through cell extension and when in close proximity to the host root and on

perception of a haustorium factor (HIF), the growth halts and cells expansion and division start to form a blug-like structure which is not specific and may attach to soil grains (Riopel and Timko, 1995; Wada *et al*., 2019). Induction of haustoria concurrently with germination minimizes contact with the host roots and abort parasitism.

HIFs are known to be derived from the host roots. In 1986 Chang and Lynn isolated 2, 6-dimethoxy-1, 4-benzoquinone (DMBQ) from sorghum roots. Further, several phenolic and structurally similar quinones and flavonoids were reported to induce haustoria in both obligate and facultative root parasitic plants (Kim *et al*., 1998, Wada *et al*., 2019). Recently phenolic compounds with one or two methoxy groups at the 3- and -5 positions and a hydroxyl group at the 4-postion induced haustoria thus indicating that HIFs originate from lignin biosynthesis and/or degradation products. Syringic acid, a phenolic acid, potentially produced from degradationof cell walls lignin's can be oxidized to DMBQ by Striga-derived H_2O_2 and peroxidases (Wada *et al*., 2019).

Conclusions

- Research in Africa on the root parasitic weed *Striga hermonthica* started since the turn of last century and several control measures were developed. However, high cost, a mismatch of the prevalent low inputs production systems, precludes their adoption.
- The present study encompassing laboratory, green house and field experiments employing three sorghum genotypes differing in their reaction to the parasite portrayed differential and temporal changes in profile of germination stimulants, haustorium factor(s) and/or inhibitors with genotype, growth stage and growth medium.
- Low haustorium initiation by root exudates of hydroponically grown Hakika and Tetron is in line with the notion that DMBQ is released through peroxidases-mediated oxidation of host lignin where host peroxidases are activated by hydrogen peroxide generated in the radicles of Striga seedlings. However, the prolific haustorium induction by root exudates of Wad Ahmed is at variance with the notion and suggests involvement of other roots exudates constituents in haustorial initiation.
- Concurrent induction of premature haustoria and germination by sorghum roots residues, though does not refute involvement of host-derived lignin in production of the haustorium factor(s), but is at variance with the notion that host peroxidases are involved in haustorium initiation and suggest that Striga radicles are capable of digesting host lignified constituents and extraction of the haustorium factor
- Low induction of germination by sorghum roots residues of field grown plants indicates that residues may have no direct practical significance in depletion of Striga seed reserves in soil, but pinpoint that concurrent

induction of haustoria with germination together with shortening of radicle could be of practical significance and may provide a novel approach for control of Striga and related parasitic Orobancheaceae

- The standard agar gel assay, though effective in identifying pre-attachment resistance based on low stimulants production, may overlook other resistance mechanisms based on post-attachment resistance.
- The study confirmed the importance of Striga seed bank in determining host damage. However, the magnitude of the damage is influenced by genotype and the measured attribute and may be related to tolerance and acclimation to the stress imposed by the parasite particularly the parasite induced perturbation of the hormonal balance.

Recommendations: -

- The plausibility of involvement of non-canonical strigolactones viz carlactone and its derivatives in Striga germination under different assay procedures and their impact on results and conclusions needs to be considered.
- Induction of premature haustoria by root residues deserves further studies as it may provide a novel approach for control of Striga and allied root parasitic Orobanchaceae.
- The plausibility of differential reaction to the hormonal imbalance instigated by the parasite, its roles in the host-parasite relationship and acclimation to the induced stress (es) need to be investigated.

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Appendices

Appendix1

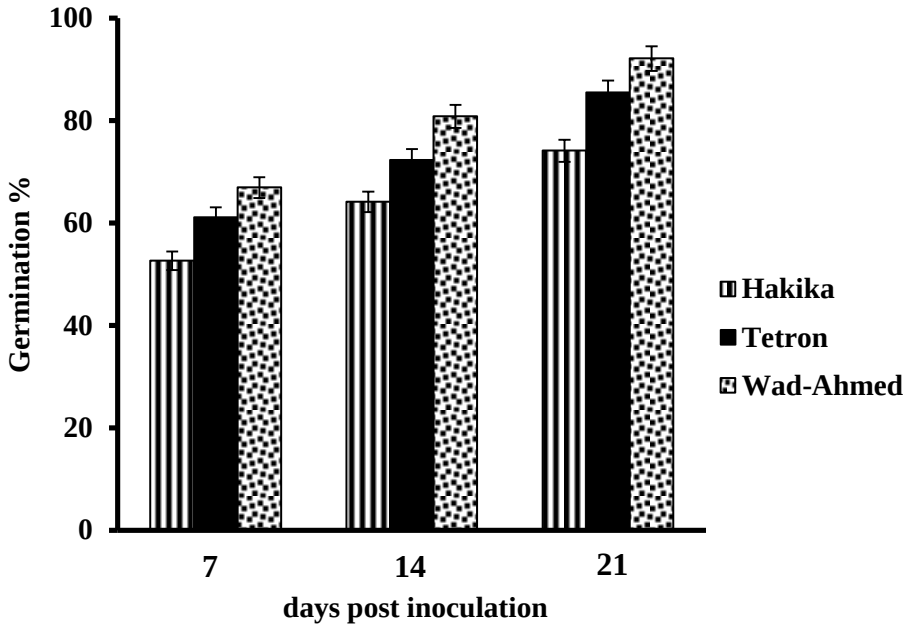

Fig.1. *Striga hermonthica*germination as influenced by sorghum genotype and time in days post inoculation. Bars, each, represent a mean of 4 replicates. Vertical bars represent standard error of the means.

Appendix2

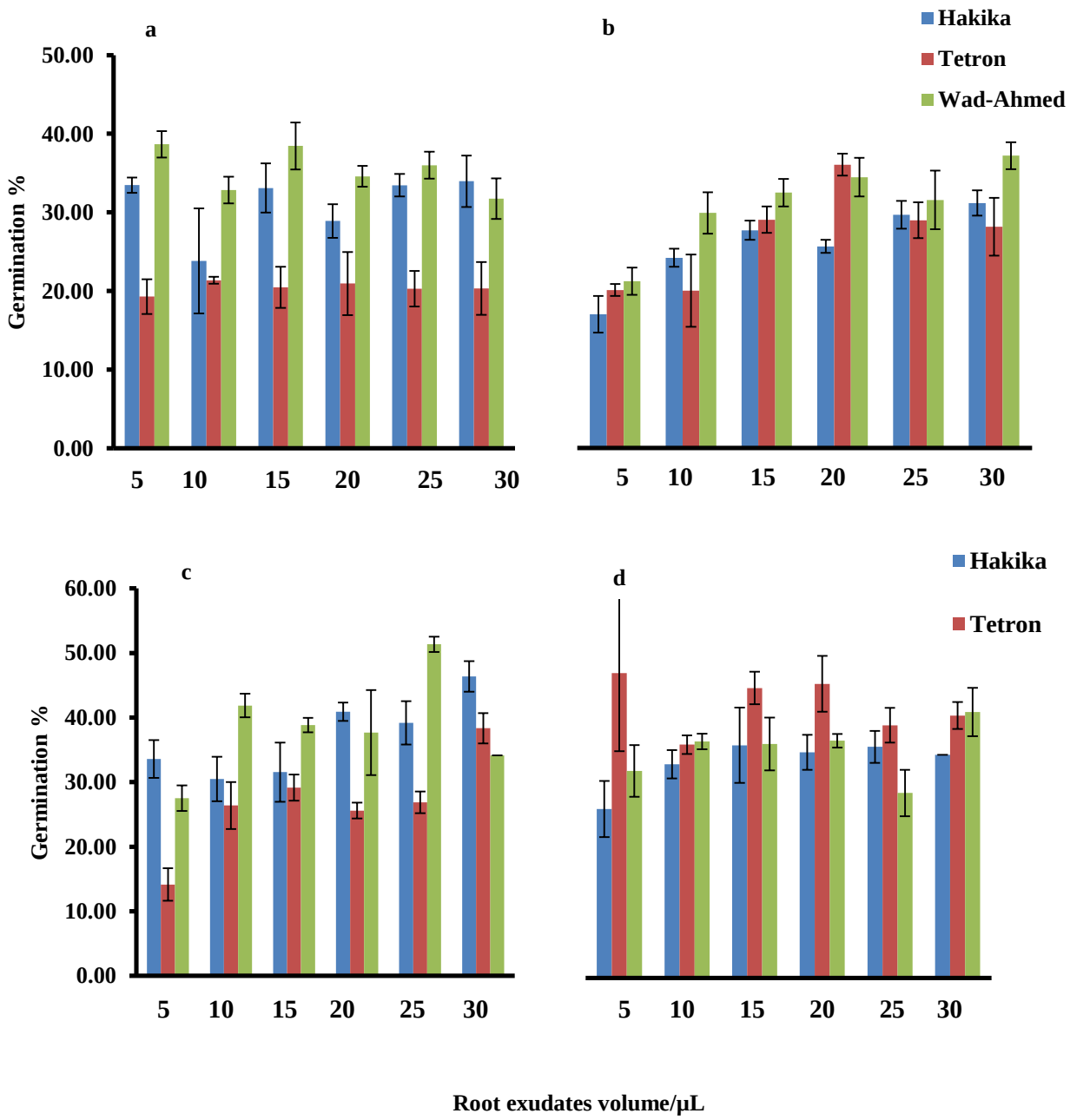

Fig.2. Effects of root exudates of hydroponically grown sorghum on germinationof *S. hermonthica* a) 7 DAS, b) 14 DAS, c) 21 DAS and d) 28 DAS.

Appendix 3

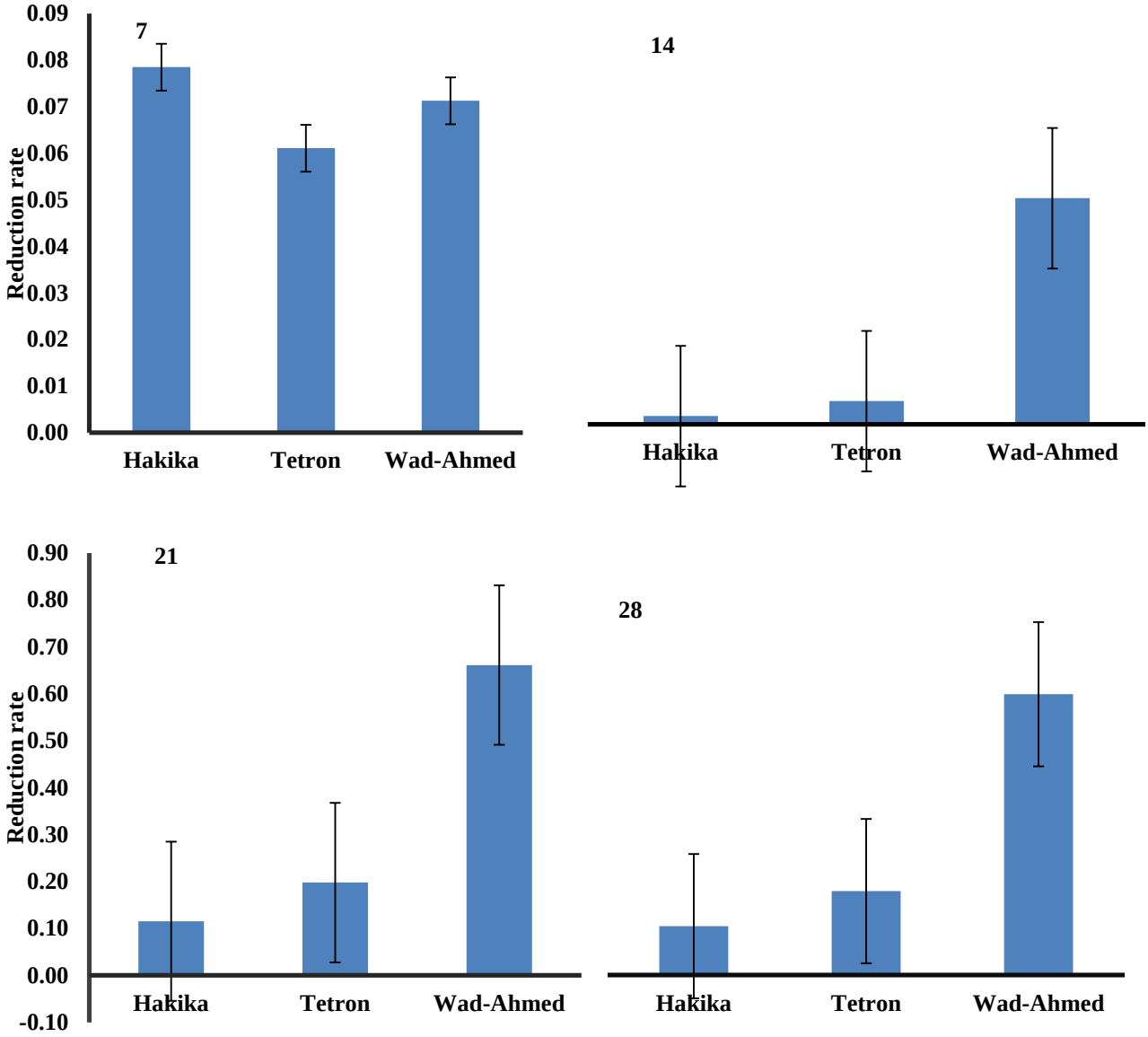

Fig.3. Effect of sorghum root exudates on *S. hermonthica* seedling radicle length at 7, 14, 21 and 28 Bars, each, represents a mean of 4 replicates. Vertical bars represent standard error of the means.

Appendix .4

Table.1. Effects of hydroponically grown sorghum genotypes root exudates on haustorium initiation in *S. hemonthica*:-

Days

Haustorium%

Root exudates volume(µL)

Cultivars	5	10	1 5	20	25	30	Mean
7 DAS							
Wad Ahmed	1.3 b	1.1 b	0.6 b	5.3 ab	2.1 b	0.0 b	**1.71 a**
Tetron	2.0 b	0.0 b	3.6 ab	12.5 a	6.3 ab	0.0 b	**4.04 a**
Hakika	2.4 ab	0.0 b	0.0 b	3.5 ab	1.3 b	0.0 b	**1.19 a**
Mean	**2.0 ab**	**0.4 b**	**1.4 ab**	**7.1 a**	**3.2 ab**	**0.0 b**	
14 DAS							
Wad Ahmed	21.0 abcd	13.2 abcd	12.9abcd	15.1 abcd	33.5 a	33.6 a	**21.39 a**
Tetron	12.2 abcd	29.0 ab	31.8 a	23.0 abc	24.5 abc	19.1 abcd	**23.24 a**
Hakika	6.8 cd	0.0 d	0.0 d	0.0 d	8.71 bcd	0.0 d	**2.59 b**
Mean	**13.3 a**	**14.1 a**	**14.6a**	**12.7 a**	**22.3 a**	**17.7 a**	
21 DAS							
Wad Ahmed	23.00 bc	16.86 cd	30.93 ab	43.39 a	34.11 ab	41.7 a	**31.7 a**
Tetron	8.33 de	0.00 e	0.00 e	0.00 e	1.19 e	0.0 e	**1.6 b**
Hakika	0.00 e	2.71 e	0.00 e	0.00 e	0.00 e	0.0 e	**0.5 b**
Mean	**10.4 ab**	**6.5 b**	**10.3 ab**	**14.5 a**	**11.8a b**	**14.0 a**	
28 DAS							
Wad Ahmed	90.6 b	97.6 ab	99.1 a	90.5 b	91.3 ab	96.1 ab	**94.21 a**
Tetron	18.1 c	7.6 de	10.1 cd	8.6 d	7.8 de	0.0 e	**8.69 b**
Hakika	3.0 de	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	**0.49 c**
Mean	**37.2 a**	**35.1 ab**	**36.4 ab**	**33.0 ab**	**33.0 ab**	**32.1 b**	

Means within a row or acolmun followed by the same letter(s) are not significantly different according to LSD at 5%.

Appendix .5

Table.2. Effects of sorghum genotype growth stage and amount on germination inducing activity of sorghum shoot powder:-

	Collection Time (DAS)					
	Germination (%)					
	Powder amount (mg)					
	20DAS					
Cultivars	**10**	**20**	**30**	**40**	**50**	**Mean**
Wad Ahmed	74.8 a	74.8 a	64.9 ab	31.3 de	12.3 fg	**43.8 a**
Tetron	31.2 de	57.8 b	60.2 ab	56.5 b	52.0 bc	**48.7 a**
Hakika	33.9 de	37.2 cd	22.1 def	26.7 def	19.1 efg	**28.8 b**
Mean	**46.6 bc**	**56.4 a**	**49.1 ab**	**38.2 c**	**27.8 d**	
	40 DAS					
Wad Ahmed	88.6 a	48.9 bcd	41.6 cde	41.1 cde	42.7 bcde	**51.7 a**
Tetron	34.4 cef	31.4 defg	51.2 bc	61.1 b	45.9 bcd	**44.8 a**
Hakika	39.3 cdef	21.4 fg	26.0 efg	24.7 efg	14.7 g	**44.1 a**
Mean	**54.1 a**	**33.9 b**	**39.6 b**	**39.0 b**	**37.8 b**	
	90 DAS					
Wad Ahmed	33.7 cde	70.6 a	31.6 cde	49.6 bc	35.2 cde	**43.7 a**
Tetron	22.5 de	18.0 e	65.1 ab	23.4 de	34.9 cde	**32.7 b**
Hakika	17.1 e	26.0 de	27.5 de	27.2 de	16.9 e	**22.1 c**
Mean	**24.4 c**	**38.2 ab**	**41.4 a**	**33.4 abc**	**29.0 bc**	
	120 DAS					
Wad Ahmed	8.9 bc	38.2 a	7.0 bc	9.5 bc	23.1 ab	**17.1 a**
Tetron	5.1 bc	7.0 bc	23.9 ab	1.8 c	9.8 bc	**10.2ab**
Hakika	5.2 bc	4.0 c	14.1 bc	3.3 c	7.4 bc	**6.8 b**
Mean	**6.4 ab**	**16.4ab**	**15.0 ab**	**4.9 b**	**13.4 ab**	

Means within a row or acolumn followed by the same letter(s) are significantly different according to LSD at 5%.

Appendix .6

Table.3. Effects of genotypes, growth stage and amount on germination inducing activity of sorghumroot powder

	Collection Time (DAS)					
	Germination (%)					
	Powder Levels (mg)					
	20 DAS					
Cultivars	**10**	**20**	**30**	**40**	**50**	**Mean**
Wad Ahmed	68.2 cd	92.7 a	84.3 abc	73.8 abcd	73.9 abcd	**76.9 a**
Tetron	65.9 cd	85.1 abc	88.2 ab	59.9 d	54.4 de	**68.7 b**
Hakika	26.5 fgh	36.6 ef	27.9 fg	18.4 fgh	9.0 gh	**21.0 c**
Mean	**53.5 b**	**71.5 a**	**66.8 a**	**50.7 b**	**45.8 b**	
	40 DAS					
Wad Ahmed	68.2 abc	82.1 a	82.2 a	62.2 abc	77.6 ab	**72.7 a**
Tetron	62.8 bc	67.7 abc	59.7 bc	49.5 cd	49.5 cd	**59.1 b**
Hakika	13.8 e	47.7 cd	35.4 d	54.4 cd	36.2 d	**33.0 c**
Mean	**48.3 bc**	**65.8 a**	**59.1 ab**	**55.4 abc**	**54.4 abc**	
	90 DAS					
Wad Ahmed	90.6 abc	73.2 cde	96.6 a	93.3 ab	84.7 abc	**87.2 a**
Tetron	76.1 bcde	83.4 abcd	96.0 a	91.9 ab	64.9 ef	**82.3 a**
Hakika	52.4 f	81.5 abcde	84.7 abc	66.5 def	51.6 f	**64.2 b**
Mean	73.0 cd	79.4 bc	92.4 a	83.9 ab	67.1 d	
	120 DAS					
Wad Ahmed	9.8 b	2.3 b	8.5 b	3.9 b	6.9 b	**6.0 ab**
Tetron	7.5 a	0.0 b	0.0 b	0.0 b	0.0 b	**1.5 a**
Hakika	0.9 b	3.6 b	1.0 b	0.0 b	1.9 b	**1.2 b**
Mean	**6.07 a**	**1.3 b**	**3.2 b**	**2.0 b**	**2.9 b**	

Means within a row or acolum followed by the same letter(s) are not significa different a ccording to LSD at 5%.

Appendix .7

Table .4. Effects of genotypes, growth stage and amount on radicle length activity of sorghumshoot powder.

	Collection Time (DAS) Radicle(µm×10^{-2}) Powder amount (mg)					
	20 DAS					
Cultivars	**10**	**20**	**30**	**40**	**50**	**Mean**
Wad Ahmed	0.18 bc	0.20 bc	0.13 c	0.30 bc	0.10 c	**0.17 b**
Tetron	0.98 a	0.53 abc	0.68 ab	0.50 abc	0.33 bc	**0.56 a**
Hakika	0.40 bc	0.43 bc	0.28 bc	0.18 bc	0.20 bc	**0.28 b**
Mean	**0.52 a**	**0.31 ab**	**0.36 ab**	**0.33 ab**	**0.21 b**	
	40 DAS					
Wad Ahmed	0.28a	0.25 a	0.23 a	0.25 a	0.23 a	**0.25a**
Tetron	0.28a	0.23 a	0.23 a	0.25 a	0.28 a	**0.25 a**
Hakika	0.33a	0.35 a	0.25 a	0.28a	0.28a	**0.29 a**
Mean	**0.29 a**	**0.28a**	**0.26a**	**0.23 a**	**0.26a**	
	90 DAS					
Wad Ahmed	0.20 abc	0.30 abc	0.33 ab	0.30 abc	0.35 ab	**0.29 a**
Tetron	0.38 a	0.30 abc	0.30 abc	0.30 abc	0.18 abc	**0.26 a**
Hakika	0.33 ab	0.25 abc	0.20 abc	0.20 abc	0.10 c	**0.21 a**
Mean	**0.30 a**	**0.28 a**	**0.27 a**	**0.26 a**	**0.21 a**	
	120 DAS					
Wad Ahmed	0.2 abc	0.15 abcd	0.23 ab	0.23 ab	0.20 abc	**0.20 a**
Tetron	0.15abcd	0.15 abcd	0.10 bcd	0.28 a	0.15abcd	**0.16 ab**
Hakika	0.10 bcd	0.13 bcd	0.05 d	0.20 abc	0.15abcd	**0.12 b**
Mean	**0.15 b**	**0.14 b**	**0.13 b**	**0.23 a**	**0.17 ab**	

Means within a row or acolum followed by the same letter(s) are not significa different according to LSD at 5%.

Appendix .8

Table .5 - Effects of genotypes, growth stage on radicle length activity of sorghumroot powder

Collection Time (DAS)						
Radicle((µm×10^{-2}))						
Powder Levels (mg)						
20(DAS)						
Cultivars	**10**	**20**	**30**	**40**	**50**	**Mean**
Wad Ahmed	0.33 a	0.25 b	0. 25 b	0.20 c	0.15 d	**0. 22 a**
Tetron	0.15 d	0.10 e	0.10 e	0.10 e	0.10 e	**0. 11 b**
Hakika	0.10 e	0.10 e	0.10 e	0.10 e	0.10 e	**0. 09 b**
Mean	**0. 19 a**	**0.15 b**	**0.15 b**	**0.13 bc**	**0.12cd**	
40 DAS						
Wad Ahmed	0.25 a	0.10 bc	0.10 bc	0.10 bc	0.10 bc	**0.13 ab**
Tetron	0.15 abc	0.18 ab	0.23 a	0.10 bc	0.10 bc	**0.14 a**
Hakika	0.08 bc	0.10 bc	0.10 bc	0.10 bc	0.10 bc	**0.09 b**
Mean	**0.16 a**	**0.13 ab**	**0.14 ab**	**0.10 ab**	**0.10 ab**	
90 DAS						
Wad Ahmed	1.15 a	0.68 abc	0.93 ab	0.40 cd	0.38 cd	**0.6 1a**
Tetron	0.93 ab	1.15 a	0.50 bcd	0.58 bcd	0.10 d	**0.56 a**
Hakika	0.10 d	0.33 cd	0.10 d	0.15 d	0.43 bcd	**0.23 b**
Mean	**0.73 a**	**0.72 a**	**0.51 ab**	**0.38bc**	**0.30 bc**	
120 DAS						
Wad Ahmed	0.05 b	0.05 b	0.10 b	0.08 b	0.10 b	**0.07 a**
Tetron	0.08 b	0.08 b	0.10 b	0.05 b	0.00 b	**0.12 a**
Hakika	0.03 b	0.03 b	0.03 b	0.00 b	0.03 b	**0.02 a**
Mean	**0.05 a**	**0.05 a**	**0.08 a**	**0.04 a**	**0.04 a**	

Means within a row or acolumn followed by the same letter(s) are not significa different according to LSD at 5%.

Appendix .9

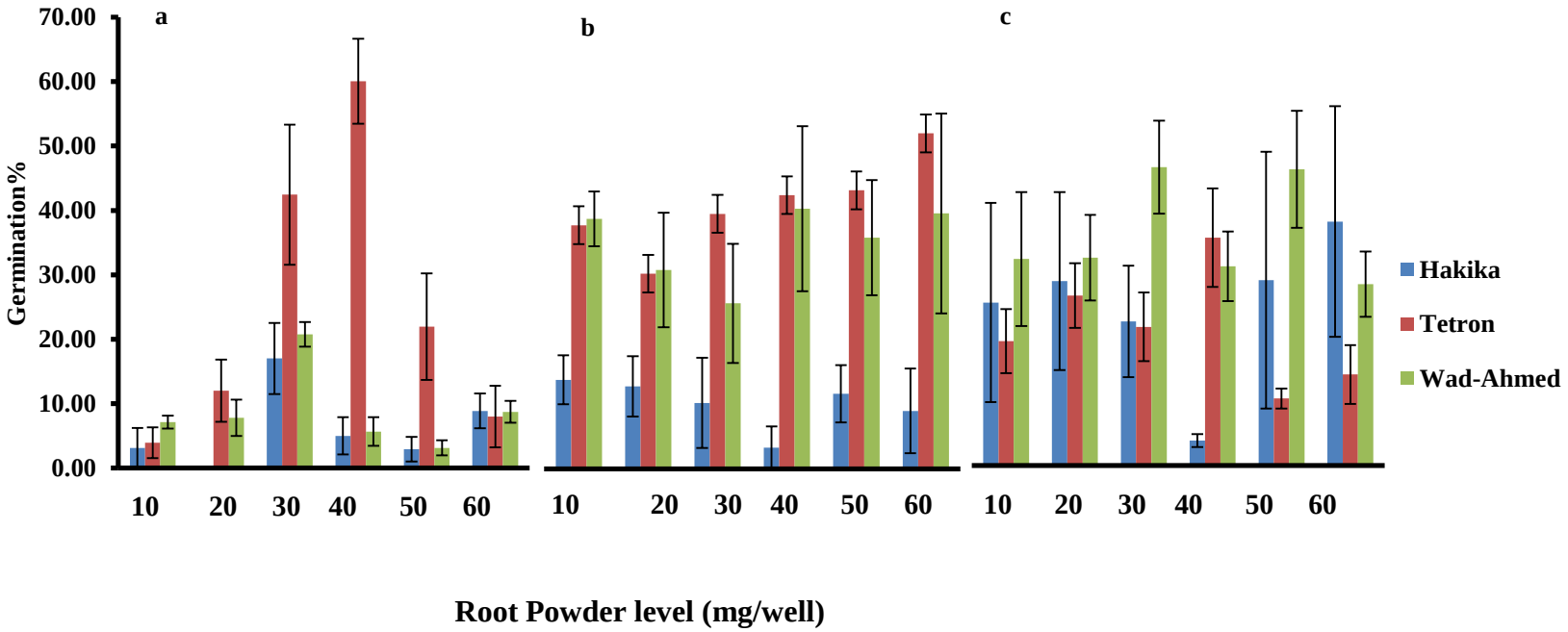

Fig.4. Effects of genotype root residues on germination of *S. hermonthica*on germination inducing activity of Sorghum root powder a) 60 DAS, b) 75 DAS and c) 90 DAS .Vertical represented stander error.

Appendix .10

Table .6. Effects of sorghum root residues on radicle length of *S. hermonthica*

	Radicle length (µm×10^{-2})						
	Days after sowing						
	60 DAS						
	Levels (mg)						
Cultivars	**10**	**20**	**30**	**40**	**50**	**60**	**Mean**
Wad Ahmed	0.02 a	0.02 a	0.02 a	0.02 a	0.02 a	0.02 a	**0.02 a**
Tetron	0.01 b	0.02 a	0.02 a	0.02 a	0.02 a	0.01 b	**0.02 a**
Hakika	0.05 cd	0.00 d	0.02 a	0.01 bc	0.01 bc	0.02 a	**0.01 b**
Mean	**0.03 b**	**0.01 b**	**0.02 a**	**0.02ab**	**0.02ab**	**0.02 ab**	
				75DAS			
Wad Ahmed	0.06 a	0.03 b	0.03 b	0.04 ab	0.04 ab	0.03 b	**0.04 a**
Tetron	0.03 b	0.03 b	0.02 b	0.03 b	0.03 b	0.02 b	**0.03 a**
Hakika	0.04 ab	0.03 b	0.04 ab	0.02 b	0.03 b	0.03 b	**0.03 a**
Mean	**0.04 a**	**0.03 ab**	**0.03 ab**	**0.03 ab**	**0.03 ab**	**0.03 b**	
				90DAS			
Wad Ahmed	0.08 cdef	0.05 ef	0.07 ef	0.08 def	0.06 ef	0.05 ef	**0.07 b**
Tetron	0.06 ef	0.10 cdef	0.05 ef	0.07 ef	0.07 ef	0.03 f	**0.06 b**
Hakika	0.25 ab	0.18 bcd	0.03 f	0.18 bc	0.31 a	0.15 cde	**0.18 a**
Mean	**0.12 ab**	**0.11 abc**	**0.05 c**	**0.11 ab**	**0.15 a**	**0.08 bc**	

Means within a row or acolmun followed by the same letter(s) are not significantly different according to LSD at 5%.

Appendix .11

Table .7. Effects of sorghum root residues on haustorial initiation *S. hermonthica*:-

	Haustorium initiation Days after sowing (DAS) 60 DAS Residues amount (mg/well)						
Cultivars	**10**	**20**	**30**	**40**	**50**	**60**	**Mean**
Wad- Ahmed	100.0 a	100.0 a	100.0 a	75.0 ab	75.0 ab	100.0 a	**91.7 a**
Tetron	37.5 bc	75.0 ab	100.0 a	100.0 a	100.0 a	50.0 abc	**77.1 a**
Hakika	25.0 bc	0.0 c	75.0 ab	50.0 abc	50.0 abc	100.0 a	**50.0 b**
Mean	**54.2 b**	**58.3 b**	**91.7 a**	**75.0 ab**	**75.0 ab**	**83.3 ab**	
			75DAS				
Wad- Ahmed	100.0 a	100.0 a	100.0 a	68.8 ab	96.43 a	100.0 a	**94.2 a**
Tetron	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	**100.0 a**
Hakika	100.0 a	75.0 ab	50.0 bc	25.0 c	75.0 ab	50.0 bc	**62.5 b**
Mean	**100.0 a**	**91.7 ab**	**72.9 b**	**75.0 b**	**90.5 ab**	**83.3 ab**	
			90DAS				
Wad- Ahmed	85.7 abc	82.1 abc	86.9 abc	98.8 a	92.5 abc	70.5 bc	**86.1 a**
Tetron	100.0 a	92.7 abc	87.5 abc	91.7 abc	100.0 a	87.3 abc	**93.2 a**
Hakika	73.3 abc	89.4 abc	65.7 c	87.5 abc	82.7 abc	94.5 ab	**82.2 a**
Mean	**86.3 a**	**88.1 a**	**80.0 a**	**92.7 a**	**91.7 a**	**84.1 a**	

Means within a row or a column followed by the same letter(s) are not significantly different according to LS 5%.