بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Susceptibility of Two Safflower (Carthamus tinctorius L) varieties to Induce Broomrapes Seed Germination, Haustorium Initiation and Radical Length

قابلية مستخلص مسحوق صنفين من القرطم في تشجيع الإنبات وتكوين الممصات وطول الجذير لنوعين من الهالوك

A Thesis submitted in partial fulfillment of the requirements for the Degree of Master (M.Sc.) in Agronomy

By: Ahmed Abdalla Ahmed Ibrahim

B.Sc. Agronomy, Omdurman Islamic University (2016)

Supervisor:

Dr: Nahid Abdalfatah Mohamed Khalel

Department of Agronomy

College of Agricultural Studies

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الآية

بسم الله الرحمن الرحيم

قال تعالى :

(إِنَّ فِي خَلْقِ السَّمَوَاتِ وَالْأَرْضِ وَاخْتِلَافِ اللَّيْلِ وَالنَّهَارِ وَالْفُلْكِ الَّتِي تَجْرِي فِي الْبَحْرِ بِمَا يَنْفَعُ النَّاسَ وَمَا أَنْزَلَ اللَّهُ مِنَ السَّمَاءِ مِنْ مَاءٍ فَأَحْيَا بِهِ الْأَرْضَ بَعْدَ مَوْتِهَا وَبَثَّ فِيهَا مِنْ كُلِّ دَابَّةٍ وَتَصْرِيفِ الرِّيَاحِ وَالسَّحَابِ الْمُسَخَّرِ بَيْنَ السَّمَاءِ وَالْأَرْضِ لَآيَاتٍ لِقَوْمٍ يَعْقِلُونَ)

سورة البقرة الآية (164)

DEDICATION

TO MY A WONDERFUL MOTHER

AND GREAT FATHER AND BROTHER

AND

TO

ALL WHOM ARE

CONTRIBUTE IN THIS WORK

THANKFUL

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Abstract

A series of laboratory experiment was undertaken at the College of Agricultural Studies, Sudan University of Science and Technology (Weeds Research Laboratory) at Shambat in 2019 – 2020, to **i)** determine the reaction of two safflower varieties (Giza and Dongla) to *Orobanche.crenata* Forsk and *Phelibanche.ramosa* and their ability to sustain successful parasitism and **ii)** ability of air dried parts and root exudates of the safflower varieties to induce germination of the parasite. The experiments showed that safflower residues, irrespective of varieties, plant parts and amount of powder, induced both germination and haustorium initiation, Dongla leaves powder gain highest germination 64.5% compared to stem powder which gave lower germination 1.1%. Giza root powder achieved lower germination 3.7% compared toleaves and stem powder 51.33 and 15.95 respectively in *O.crenata. P.ramosa* affected significantly by Giza root powder which gave 61.2%, an increase in leaves powder level (10, 20 and 40 mg) showed high decreased in germination 11.32,8.8 and 9.19 % respectively.

Giza and Dongla varieties, powder induced haustorium initiation and decreased radical length. Leaves powder displayed better haustorium initiation than other parts of the plant in broomrape. Giza showed high significant compared to Dongla.

Root exudates results showed that Giza and Dongla varieties induced higher germination at the third week after germination and then start to be lower after fourth week after germination. Giza 7, 14, 21 and 28 days displayed 17, 22, 30, and 8 52% germination respectively, compared to Dongla which gained 22, 43.40, 28 and 35% germination at the same period respectively in *O.crenata*.

In *P.ramosa,* Giza at 7, 14, 21 and 28 days displayed 12.15, 17.4, 18 and 15% germination respectively and Dongla recorded 7.42, 23.4, 18.6 and 30%

germination respectively at the same period. Results showed that Giza and Dongla varieties susceptible to support germination.

الملخص

أجريت دراسة معملية في كلية الدراسات الزراعية (شمبات) بجامعة السودان للعلوم والتكنولوجيا في العام 2019–2020 لتحديد (1) تفاعل صنفين من القرطم في تشجيع التطفل الناجح لنوعين من الهالوك (2) مقدرة اجزاء القرطم المجففة هوائياً ومستخلصات الجزور في تشجيع انبات طفيل الهالوك. أظهرت التجارب المعملية أن بقايا القرطم، بغض النظر عن الأصناف، أجزاء النبات وكمية البودرة، تعمل علي تشجيع الإنبات وتكوين الممصات، حقق مسحوق اوراق الصنف دنقلا أعلى معدل تحفيز حوالي 64.54% مقارنة بمسحوق الساق الزي اعطى اعلى معدل انخفاض 1.08%، اما مسحوق جزور الصنف جيزا اعطى اعلى متوسط انخفاض حوالي 3.07% مقارنة بالأوراق والساق 51.33 و15.95% على التوالي علي النوع *O.crenata*، ابانت النتائج ان مسحوق جزور الصنف جيزا على النوع *P.ramosa* أعلى متوسط تحفيز نمو حوالي 61.22%، اما بزيادة مستويات مسحوق الأوراق من 10 الي 20 و40 ملجم, أظهر أعلى متوسط إنخفاض نمو حوالي 11.32، 9.19 و8.08% على التوالي. مسحوق الصنفين معاً حفز تكوين الممصات وخفض طول الجذير، مسحوق الأوراق اعطى اعلى متوسط تكوين للممصات من الأجزاء الأخرى في النبات على الطفيلين معاً، جيزا اظهر اعلى مستوى معنوية مقارنه بدنقلا.

اظهرت نتائج مستخلصات جزور اصناف القرطم جيزا ودنقلا المزروعة مائيا تشجيع وتحفيز في انبات الهالوك بلغ زروته في الإسبوع الثالث وإنخفض نمو الطفيلات، الممصات وطول الجذير في الاسبوع الرابع بذيادة حجم الإفرازات الجزرية وكانت نسب الانبات على النوع *في* 7 ,14 ,21 ,28 يوم اعطت نسب انبات حالي 52 ,30.8 ,22 ,17% علي التوالي عبر الصنف جيزا، مقارنة بدنقلا الزي اعطى نسب انبات حوالي 35 , 14 ,43.40 ,22% علي التوالي في نفس الفترة في طفيل النوع *O.crenata*.

اما على طفيل *P.ramosa* في 7 , 14 ,21 ,28 يوم اعطت نسب انبات حوالي 15 ,18 ,17.4 ,12.15% علي التوالي عبر الصنف جيزا مقارنة بالصنف دنقلا الزي اعطى نسب انبات حوالي 30 , 18.6 ,23.4 ,7.42% علي التوالي في نفس الفترة. خلصت الدراسة بأن صنفين القرطم جيزا ودنقلا قابلا للإصابة بطفيل الهالوك ولهما المقدرة علي دعم الانبات.

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List of Abbreviations

%	Percent
^{o}C	Degree centigrade
µM	Micro molar
Mm	Mille molar
µl	Micro liter
Fig	Figure
GR24	*Orobanche* Synthetic germination stimulant
Ppm	Part per million
G	Gram
Mg	Milligram
SE	Standard error
H	Hours
Ha	Hectare
LSD	Less significant difference
Min	Minute
GFFP	Glass fiber filter papers
et al	And others

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CHAPTER ONE

INTRODUCTION

Parasitic weeds of the families Orobanchaceae (*Aeginetia, Striga, Orobanche*,) and Scrophulariaceae (*Alectra*, witchweed) are considered to be among the most serious agricultural pests of economic importance in many parts of the world (Joel., 2007).

Parasitic weeds (over 4000 species) represent one of the most destructive and intractable problems to agricultural production, causing heavy damage to numerous crops, reducing both crop yield and quality. The Orobanche species (broomrapes) are obligate root parasites of important dicotyledonous crops in semiarid regions of the world. Unlike other weeds, these parasitic weeds are directly connected through haustoria to the vascular system of crop plants that serve as their hosts, and the parasite becomes a major sink for crop photosynthes, debilitating crop growth and yield up to a total loss. Each mature Orobanche plant produces tens of thousands of seeds, which can remain viable in soil for many years. Seeds germinate only after receiving a chemical stimulant from a neighboring host root (Parker and Riches., 1993).

Orobanche crenata, (crenata broomrape) is an important pest of most grain and forage legumes being widely distributed in the Mediterranean basin and Middle East (Rubiales *et al*., 2009). *Orobanche minor*, is widely distributed but of economic importance on clover (*Trifolium sp*.) only (Eizenberg *et al*., 2005). *Phelipanche aegyptiaca* (Pers.) *Phelipanche ramosa* (L.) is an important pest of legumes but also of many vegetable crops especially in semi-arid regions of the world (Parker, 2009). There is no single technology to control these parasitic weeds (Parker 2009; Rubiales *et al*., 2009). The only way to cope with parasitic weeds is through an integrated approach, employing a variety of measures in a

concerted manner, starting with containment and sanitation, direct and indirect measures to prevent the damage caused by the parasites, and finally eradicating the parasite seed bank in the soil. Few practical and economically sound methods are available for controlling parasitic plant species (Gressel *et al*., 2004; Rispail *et al*., 2007), in part because their physiological connection to host plants limits the usefulness of most herbicides. Parasitic weeds can also be difficult to eradicate because they often produce large numbers of long-lived seeds. For example, a single *Orobanche* sp. plant can produce over 200,000 dust-like seeds that remain viable for 8–10 years (Parker and Riches, 1993). In addition, parasitic plants that attack host roots can inflict serious damage to crop plants before the latter emerge from the soil, making it difficult to diagnose infestations before economic losses occur, as sunflower varieties are resistant to *O.crenata* (serghini *et al.*, 2001) and more recently unidentified safflower appeared to inhabit seed germination of Orobanche when intercropping with crops.

Safflower *(Carthamus tinctorius* L), is one of the world's oldest crops belongs to the Asteraceae family (Bérvillé *et al*., 2005). Common names with countries recorded include: 'safflower' , 'Zaffrone' , 'Hung' , 'Alazor' , 'assfore' , 'saffron' , 'Qurtom ' , 'kusum' is one of oldest annual oilseed crops, which occurs in Mediterranean region, (Srinivasa *et al*., 2017). From the genetic source point of few Iran have a rich genetic source of safflower in the world. Safflower cultivation was done commonly in many regions of eastern hemisphere especially in middle east as it was cultivated in Egypt since 3500 years ago (Omid Beigi.,1997).

Traditionally, safflower was mainly grown for its flowers, which were used as dye sources for coloring foods and textiles as well as medicinal purposes (Bagheri and Sam-Dalini, 2011). Nowadays, this crop cultivated mainly for its seed, which is used as edible and industrial oils and as birdfeed. Although

safflower ranks last among the oil seed crops, it includes some voluble characteristics that have made the species famous throughout the centuries, in particular as a multi-purpose oilseed (Gecgel *et al*., 2007). By product which remains after producing oil from the seeds it is very nutritional as an animal and birds feedings, it contains about 25% protein and carbohydrate as a source for energy (Hume., 1995**)**.

Safflower was not cultivated in Sudan, except in the Northern State along the River Nile, although cultivated safflower is believed to have originated from here (Northern Sudan bordering Egypt) (Knowles *et al*., 1989). It had been grown for a long time, under small areas in the Northern State for farmers' personal use, but detailed records are not available.

The research development on different aspects of safflower despite its adaptability to varied growing conditions with very high yield potential and diversified uses of different plant parts, have not received attention this probably is the main reason for its status as a minor crop around the world in terms of area and production compared to the other oilseed crops. However, interest in this crop has been rekindled in the last few years due to its multi usage.

Due to the observation of the presence of O.*crenata* in the field of Safflower in previous experiment at the College of Agriculture Studies, there for the work is designed to investigate the following objectives:

i). To study the effect of air dried parts of the safflower powder to induce seeds germination, haustorium initiation and radical length of the parasite ii) To determine the reaction of safflower varieties root exudates to *O.crenata* and *P.ramosa* seed germination and their ability to sustain successful parasitism

CHAPTER TWO

LITERATURE REVIEW

2.1. Botanical description

Safflower (*Carthamus tinctorius* L), belongs to the family compositae or Asteraceae, genus carthamus the cultivated plant has a chromosome number of 2n=24 (Ekin, 2005). The crop is an annual broad-leaved plant, it grows well in both dry land and irrigation areas. It is a drought-tolerant plant, Safflower is thistle-like, with a main stem and a number of branches. It stands 1 to 4 feet tall at maturity. Its taproot can penetrate 8 to 10 feet depending on subsoil, temperature and moisture. The flowering period lasts 4 to 6 weeks depending on the culture practices and climatic conditions especially temperature. Shades of orange, yellow and red flower are produced, the flower are tubular and largely self-pollinating with generally less than 10% cross-pollinating (Emongor, 2010).

Each plant produces a number of flower heads, flower petals are red, white, yellow, or orange. Each head contains 20 to 100 seeds in glossy white, brown, or white with gray, black, or brown stripes color. On average, safflower is ready to harvest about 35 to 40 days after the peak of flowering (Emongor., 2017).

2.2. Economic importance

The crop was a relatively considerable oilseed crop until the early 1950s when higher yielding genotypes were released and it was considered as a source of oil for surface contain (O'Brien., 2009) This oil lowering the cholesterol level in the blood, protect body tissues and protect interior organ (Fasina *et al*., 2006). The oil is heat-stable; therefore, it is used as cooking oil. Moreover, safflower oil is used in food coating and infant food formulations. Furthermore, the oil is used in salad dressing and for the production of margarine. The flowers are occasionally

used in cooking as substitute for saffron (Bergland *et al*., 2007).Also it use as a cut flower, vegetable and Medicinal plant. (Ekin, 2005). Safflower derived human insulin. The Insulin (SBS-1000) that was extracted from safflower plants and was created by Sem biosys, (2006) has been injected into people for first time. The hope is that plants will provide a cheaper source of insulin for people with diabetes (SemBiosys, 2006). All essential amino acids except tryptophan were present in safflower flowers (Singh, 2005). The crop has become an industrial crop production, a different appearance from other oilseed crops and excellent agronomic traits such as taproot architecture that accesses sub-soil water reserves (Markley *et al*., 2006).

Another important and interesting use of safflower seed has recently emerged by means of its genetic modification to produce high-value proteins as pharmaceuticals and industrial enzymes. SemBioSys transforms safflower tissue genetically in order to get the proteins of interest to accumulate in the seed of the mature transgenic plant (Mundel *et al.*, 2004).

Safflower also makes an acceptable livestock forage if cut at or just after bloom stage (Bergland *et al*., 2007). Safflower hay, given adlibtum, has been successfully used as a sole feed for late-pregnancy dairy cows (Landau *et al*., 2004).

2.3 Climatic requirements

Safflower is not suited to lowland, humid tropics. Large scale commercial cropping is practiced in USA and USSR between 30º and 45ºN and in Australia between 15º and 35ºS. Emerging plants need cool temperatures for root growth and rosette development mean daily temperature 15 to 20ºC and higher temperatures during stem growth, flowering and yield formation periods 20 to 30ºC. There is no germination below 2-5ºC; germination takes 16 days and at

16ºC, 4 days. The seedling is frost-resistant up to -7ºC but after this stage frost below -2ºC kills the plant. The crop seems to be sensitive to day-length .The length of the growing period for an autumn-plant crop varies from 200 to 230 days; when plant in spring, 120 to 160 days (FAO, 2013).

The crop grows well in well-drained, deep, fertile, sandy loam soil. In heavy clay soils, crusting may reduce seedling emergence. In general, if soil moisture is limiting, good irrigation just prior to bloom increases seed yield significantly (Siddiqui and Oad, 2006).

2.4. Parasitic plants

Over 4100 species, belonging to 19 families of flowering plants, are able to directly invade and parasitize others plants (Nickrent and musselman, 2004). However, very few parasitize cultivated plants, nevertheless, weedy parasites pose tremendous threat to the world economy, mainly, because they are almost uncontrollable (Parker and Riches, 1993, Gressel *et al*., 2004). Among parasitic weeds those of the Orobanchaceae received considerable attention because of interest for evolutionary studies as it encompasses closely related species with vast difference in host range (Parker and Riches, 1993).

Parasitic plants form a close connection with the vascular system of their host plant(s) through a specialized structure known as a haustorium (plural haustoria), that physically connects the parasite to the host, providing a vascular conduit for water and nutrients (and sometimes sugars and amino acids) from host to parasite. They are dependent on their host for their supply of mineral/inorganic nutrients, water and/or organic compounds, although the degree of host dependency varies greatly between species. Almost 1% of the world's flowering plants are parasitic (3,000 species), and it is estimated that parasitism has

appeared at least 11 times during the evolution of angiosperms (Barkman *et al*., 2007).

Broomrapes (*Orobanche* and *Phelipanche*) form a large group of root-holoparasites. Flowering plants that occur mainly in the South-East of Europe, West Asia and North Africa (Parker and Riches, 1993). The majority of broomrape species has a narrow host range and grows on perennial host plants. These species are usually found in natural ecosystems (Teryokhin, 1997).

The Mediterranean region is considered to be one of the centers of origin of *Orobanche* species. The species are distributed worldwide from temperate climates to the semi-arid tropics. The distribution of Orobanche *crenata* Forsk. Is restricted to the Mediterranean regions, the Middle East and East Africa (Ethiopia), while other species have a wider spread. Today, the species *O. crenata*, *P. ramosa* L., *O. aegyptiaca* Pers., *O. cernua* Loefl., *O cumana* Wallr., *O. minor* Sm. and *O. foetida* Poir. are one of the major biotic limiting factors of the production of legumes such as faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medick.), and to crops of the family Solanaceae Tomato (*Lycopersicon esculentum* Mill.), Potato (*Solanum tuberosum* L.), Tobacco (*Nicotiana tabacum* L.)] and Asteraceae, mainly Sunflower (*Helianthus annuus* L.) (Parker and Riches, 1993).

2.5. Economic importance

Parasitic plants, in general, lead to losses in productivity of their hosts. However, few of them are considered as agricultural pests. Of all parasitic plants those of the family Orobanchaceae are the most important (Babiker, 2007). *Orobanche* and *Phelipanche* species have become weedy, having specialized to parasitize annual crops in human disturbed ecosystems. These species are responsible for high crop production loss, from 5 to 100% (Joel *et al*., 2007; Schneeweiss,

2007). Among all identified weedy broomrapes, distinct patterns of host specificity have been reported. For example, *Orobanche cumana* preferentially parasites sunflower (Echevarría-Zomeno˜ *et al*., 2006; MolineroRuiz *et al*., 2008). The effects of the parasite on crops ranges from stunted growth through wilting, yellowing, and scorching of leaves, to lower yield and death of the infected plant (Gressel *et al*., 2004).

In Sudan *O. crenata* was first reported in 2000/2001 as minor pests on horticultural crops and common. Of the four *Orobanche* species *P.ramosa* and *O.crenata* are naturalized and have become pests of economic importance .Tomato (*Lycopersiction esculentum* Mill.) and potato (*Solanum tuberosum* L.), are the main hosts of *p. ramosa*, on 40, 20 and 66 000ha, respectively mainly in the alluvial soils of the Nile valley. The host range of *O. crenata* is somewhat narrower than that of *P. ramosa*, with the main host crops restricted to the family Fabaceae and Umbelliferae (Apiaceae) and relatively sporadic occurrence on crops in a few other families such as Compositae (Asteraceae) and Cucurbitaceae. Wild hosts are mainly in the same families.

Mismanagement stemming from lack of awareness of the debilitating effects of the Orobanche species their biology invasive nature and means and methods of spread has led to out-break of the parasites. Broomrape weeds (Orobanche and Phelipanche species), are root-holoparasitic plants that possess extreme competitive ability against the crop, rather than to compete with crops for field resources, their haustorial cells penetrate crop roots to directly divert water and nutritive resources (Parker and Riches, 1993).

2.6. Broomrape biology and physiology

O. crenata is holoparasite plant, needing to establish a connection to a host root within a few days of germination. *Orobanche* spp. depends totally on their hosts for all nutrition, and draws most of their water from the host root. Effects on the host are directly proportional to the biomass of the parasite, such that the mass of the parasite is reflected in a very similar loss in mass of the host crop (Manschadi *et al.*, 1996).

The *O. crenata* seed is approximately 0.2 x 0.4 mm in size, from which only the radical emerges and this can grow only a few mm long. A chemical stimulus is needed to initiate Orobanche germination. This stimulus normally comes from host roots. However, a moist environment is required (for several days), together with suitable temperatures, before the mature seed is responsive to germination stimulants. This preparatory period is known as conditioning or preconditioning. Conditioned seeds remain responsive to germination stimulants for several months. This combination of characteristics ensures that the seeds have a characteristic cycle of germinability through the year and tend to germinate freely over a relatively short period in early to mid-winter. On contact with the host root, a swelling, the haustorium, is formed and intrusive cells penetrate through the cortex to the vascular bundle to establish connection with the host xylem and phloem (Dorr and Kollmann, 1995).

2.7. Life cycle of *Orobanche spp*

The life cycle of these parasitic weeds starts with seed germination in response to strigolactones which are exuded by roots of host and non-host plants and act as germination stimulants (Bouweester *et al*., 2007). Following seed germination radicles elongate, from haustoria penetrate the host root and fuse with vessels of the host vascular cylinder after the successful establishment of xylem connection

in seed (Parker and Riches, 1993). The parasites are capable to generate a huge number of seeds that may stay viable in the soil for more than 20 years (Aly, 2007; Lopez Raez *et al*., 2009).

Upon germination a germ tube, which in close proximity to the host roots, elongates towards the root of the host, develops an organ of attachment the haustorium which serves as a bridge between the parasite and its host and deprives it of water, mineral, nutrients and carbohydrates causing drought stress and wilting of the host. Stunted shoot growth leaf chlorosis and reduced photosynthesis are also phenomena that can be observed on susceptible host plants which contribute to reduction of grain yield (Frost *et al*., 1997).

Most of the seeds in the soil will not be reached by the stimulant but will remain viable for up to 15 years forming a seed reservoir for the next cropping season. The penetration of haustorial cell into host tissue (zylem and/or phloem system) is carried out mechanically by pressure on the host endodermal cells and by hydrolytic enzymes. Conditioning germination parasitic contact (attachment) and penetration are mediated by elegant systems of chemical communication between host and parasite (Maass1999). After several weeks of underground development parasite emerges above the soil surface and starts to flower and produce seeds after another short period of time. Seed production is prodigious u to 100000 seeds or even more can be produced by a single plant and lead to a re-infestation of the field. Thus, if host plants are frequently cultivated the seed population in the soil increases tremendously and cropping of host plants becomes more and more uneconomical (Kroschel, 2001).

2.8. Control methods:

Compared with non –parasitic weeds, control of parasitic weeds has proved to be exceptionally difficult (Parker and Riches, 1993, Babiker, 2007). The ability of the parasites to produce a tremendously high number of seeds, with prolonged viability and intimate physiological interactions with their host plants, are the main obstacles that limit the development of successful control measures that can be accepted and used by subsistence farmers (Elzein and Kroschel, 2003). However, several methods have been tried for the control of parasitic weed, including preventive, cultural biological and chemical measures. So far these methods, have only limited impact on the parasitic and up to-date there is no single control method that can effectively solve the problem to control them (Gressel *et al*., 2004).

2.9. Preventive methods

The durability of broomrape lies in its ability to form a bank of seeds in the soil. A management or eradication program must aim at reducing this seed bank, while reducing the production of new seeds and their dispersal to new sites. Quarantine is therefore an essential element in control or eradication programs. The best option for winning against broomrapes is avoiding the fight. It is impossible when the fields are already infested with the seeds, but preventive measures must be taken into consideration to avoid distributing the infestation into neighboring fields (FAO, 2008). The dispersal of broomrape seeds are through machinery and tools, and together with the host seeds; thus proper phytosanitary measures in and around the field are necessary to minimize the spread of *O. cernata.*

Farm equipment and machinery should be cleaned prior to their use in un-infested fields. Special care must be applied to disinfestations and cleaning of

field machinery and harvesters, and avoid trucks going from infested to non-infested fields. Containment is necessary to avoid spreading of the infestation and eradication programs should be considered. Orobanche, shoots should be removed prior to flower opening. The collected shoots should be burnt or disposed of properly. Good extension agents could easily convince the farmers to execute such task, especially when they made aware of the tremendous production of Orobanche seeds per plant.

One important spreading agent of various weeds, including Orobanche, is the uncontrolled movement of grazing animals. Which should be forbidden to enter un-infested fields after grazing infested areas (Panetta and Roger, 2005). Furthermore, farmers should use certified seed in order to ensure themselves it is clean of parasite seeds. Strict quarantine measures, at various levels, national and international, help in preventing the introduction of the parasite into parasite-free areas. Technical inspection of imported agricultural materials should be carried out by a subject matter specialist (FAO, 2008).

2.10. Cultural methods

It is traditional methods of weed control that can be practiced by many farmers in developing countries; this includes crop rotation, trap cropping, intercropping, catch cropping, hand pulling, weeding, planting date, fertilization and deep plowing, will gradually decrease parasitic weeds infestation. But complete control will be achieved after a very long period, due to the long life-span of parasitic weed seeds (Megersa Kebede and Bogale Ayana., 2018).

2.10.1. Planting date

The change of the sowing date seems not to be very promising due to uncertainty of the environmental conditions, specifically temperature and rainfall situation.

Early plantings of cool season legumes are more severely infected by parasitic weeds. Delayed sowing is the best-documented traditional method for *O. crenata* avoidance (Pérez-de-Luque *et al*. 2004; Grenz *et al*., 2005). It also reduces S. gesnerioides and dodder infection (Mishra *et al*., 2007).

2.10.2. Plowing

Plowing of the soil can strongly affect the parasitic weed seed bank. Minimum tillage can contribute to parasitic weed control by reducing the amount of viable seeds incorporated into the soil (Ghersa and Martínez-Ghersa., 2000). On the other hand, deep-ploughing has been recommended to bring the seeds into a depth, where they cannot germinate due to the lack of oxygen (Van Delft *et al*., 2000).

2.10.3. Hand weeding

Hand weeding can only be recommended in cases of limited infestation to prevent any further increase in the parasite population and to reduce the seed bank in the soil. However, even when hand weeding is still commonly used in some countries where no other feasible means of control are available and the wages for labor are cheap, it is only practical in preventing build-up of parasite seeds in slightly infested soils (Rubiales *et al*., 2003).

2.10.4. Trap cropping

Trap crops are commercially valuable crops that are able to reduce the seed bank of parasitic plants. They are false hosts owing to their ability to fool the parasite by triggering germination but not being compatible in the downstream infection process. Promising results for trap crops have been reported for many host parasite interactions including pepper (*Capsicum annuum*) against *O. aegyptiaca* and *O. cernua* LoefI Barley, common vetch, corn (Zea mays), oats (*sativa*),

sugar pea (*Pisum sativum*), sunflower, tomato and wheat against *P. ramosa* (Lins et al., 2006).

2.10.5. Catch crop

Catch crops in contrast to trap crops are plants which are heavily parasitized. Harvesting or destroying them after the appearance of the parasite would dramatically reduce the seed potential in the soil. The ideal solution would be a catch crop of agronomical interest by itself (Fernandez-Aparicio., 2012). On other hand, Link *et al* (1993) reported a 30% reduction in the broomrape seed bank after one catch crop cycle.

2.10.6. Intercropping

Intercropping is already used in regions of Africa as a low-cost technology of controlling parasitic plants (Oswald *et al*., 2002). And it's a method for simultaneous crop production and soil fertility building (Chaibi *et al*., 2008). It has been demonstrated that intercrops with some plants can increase crop production and soil to reduce the infection by parasite (Oswald., 2002).

2.10.7. Rotation

Rotation may have direct and indirect impacts on parasitic weed in infested areas. While trap- and catch-crops in rotation may reduce to some extent the parasite seed bank in soil, other rotation crops may have allelopathic effects on parasitic weed seeds. Decreasing host cropping frequency cannot, by itself, solve the parasitic weed problem. A nine-course rotation would be required to prevent *O. crenata* seed-bank increases (Grenz *et al*. 2005).

2.10.8. Nitrogen Fertilization

Nitrogen compounds and manure fertilization has potential for control of broomrape species. Nitrogen in ammonium form affects negatively root parasitic

weed germination (Van Hezewijk and Verkleij, 1996). In addition, manure fertilization augments the killing effect of solarization on *O. crenata* seeds (Haidar and Sidhamed., 2000).

2.11. Physical methods

2.11.1. Flooding

Flooding requires the availability of water which is scarce in the Near East countries. Flooding for an extended period kills the parasite seeds in the soil. Continuous irrigation or flooding was found to reduce the problem of parasitic plants, Flooding practice proved useful in some countries, where host crops are planted after rice (*Oryza sativa*). *Orobanche* infestation was drastically reduced in such rotation (Abu-Irmaileh, 2003).

2.11.2. Soil Solarization

Soil solarisation has been proven as the most effective method in controlling broomrape in open crops fields (Haidar and Sidahmad, 2000). It means heating of soil by sunlight trapped under a mulch of black, or more usually clear, polyethylene film. The temperatures of 48-57 °C kill Orobanche seeds that are in the imbibed state; therefore, soil must be wet at the time of treatment. This technique has been used successfully on croplands in many countries around the world like Middle East with an endemic Orobanche problem, as a pre-planting treatment for tomato (*Lycopersicon esculentum* Mill.), carrot (*Daucus carota* L), eggplant (*Solanum melongena* L), faba beans (*Vicia faba* L) and lentils (*Lens culinaris*). As global environmental quality considerations grow in importance, along with an increasing human population, evolving concepts, such as soil solarization and other uses of solar energy in agriculture, will become more important (Ashrafi *et al.*, 2009).

2.12. Chemical control

2.12.1. Germination stimulants

The use of chemicals with the ability to stimulate broomrape seed germination in the absence of a suitable host may lead to a reduction in the *Orobanche* seed bank. This is "suicidal" germination because; once germinated, the *Orobanche* seeds cannot return to dormancy and cannot survive for longer than a few days without nutritional supply from a host. This is an ideal control strategy, but its utility is limited in real-world applications.

Initial attempts to deplete broomrape seed banks using synthetic Strigolactone were made by using the synthetic Strigolactone analogue GR7. However, field application of these GR type Strigolactones provided only partial control of Orobanche to the instability of the compound particularly at pH >7.5 (Fernandez-Aparicio *et al.,* 2011). New Strigolactone analogues are continuously being produced (Mwakaboko and Zwanenburg, 2011), and these could serve as candidates for the suicidal germination approach, as long as they can be properly formulated for field application.

Although trans-22-dehydrocampesterol stimulates *O. aegyptiaca*, *O. crenata*, *O. foetida,* and *O. minor,* Soyasapogenol B was very specific stimulating the germination of *O. minor* seeds only (Evidente *et al.*, 2011).

Applying the gibberellin inhibitor uniconazole to soil near sunflowers significantly decreased broomrape parasitism and increased sunflower performance (Joel, 2000). Sunflower varieties that are resistant to *O. cernua* exude coumarins that inhibit germination and are toxic to newly germinated seedlings (Serghini *et al*., 2001). More recently, unidentified allelochemicals from oats appeared to inhibit seed germination of *O. crenata* and reduced parasitism when intercropped with legumes (Fenandez´-Aparicio *et al*., 2007).

Seed germination can also be influenced by some amino acids, which have been shown recently to have profound effects on the development of *P. ramosa*. For instance, applying exogenous methionone almost completely inhibited seed germination and reduced the number of developing Orobanche. tubercles on tomato roots, possibly indicating that soil applications of amino acids or amino acid-producing microbes might be used to manage parasitic weeds (Vurro *et al*., 2006).

2.12.2. Herbicides

The herbicides that are currently in use for broomrape control are glyphosate, and herbicides belonging to the imidazolinones (Eizenberg *et al.*, 2006) or sulfonylureas. Imidazolinones and sulfonylurea herbicides inhibit acetolectate synthesis, they are systemic herbicides absorbed through foliage and roots of plants with rapid translocation to the attached parasite, which acts as a strong sink (Colquhoun *et al.*, 2006).

glyphosate could be effective for broomrap control only on a few hosts in the family's‟ *Apiaceae, Fabaceae* and *Brassicaceae.* However, under favorable environmental conditions for broomrape attack, the treatment must be supplemented to obtain high broomrape control (Sanchez *et al.*, 2003).

2.13. Biological methods

Biological control of weeds with insects and microbial agents means the utilization of living organisms to manipulate (suppress, reduce, or eradicate) weed densities. Biological control, especially using insects and fungal antagonists against parasitic weeds, has gained considerable attention in recent years and appears to be promising as a viable supplement to other control methods.

2.13.1. Insects

Many phytophagous insects have been collected on Striga and Orobanche species but most are polyphagous and the target weed species are often not their principal host plants. (Kroschel *et al.*1999; Traoré *et al.*1999). The fly *Phytomyza orobanchia* Kalt. (Diptera: Agromyzidae) and *Smicronyx cyaneus* Gyll, are of great interest in biocontrol of Orobanche spp. (Klein and Kroschel, 2002).

As a single method, biological control with herbivores will hardly be fully successful in tackling the parasitic weed problem. However, also classical biological control might be an option by introducing *P. orobanchia* into countries where Orobanche spp. are not endemic and have been unintentionally introduced, such as Chile (Klein and Kroschel, 2002).

2.13.2. Microbial agents

Plant pathogens are proposed for use in a non-classical inundative approach as 'bioherbicides' for biological control of parasitic weeds. The protocol for their use involves: to survey the weed for pathogens; isolation; identification and classification; inoculum production; screening for efficacy (pathogenicity testing); host specificity and safety testing; inoculum mass production; preliminary field testing; formulation and delivery to target weed. Accordingly, a variety of fungal and very few bacterial agents applied to the seeds, foliage and/or soil have been explored as potential candidates for parasitic weeds of the genus Striga and Orobanche since the early 1990s (well reviewed in Kroschel and Müller-Stöver, 2003).

Müller-Stöver, (2001) observed a reduced germination rate of *O. crenata* seeds caused by FOO, although no pathogenicity was observed towards later developmental stages of the parasitic weeds. FOXY and FARTH, isolated in

Israel attack *O. aegyptiaca, O. crenata* and *P. ramosa*, but are avirulent against *O. cumana* (Amsellem *et al*. 2001).

2.15. Integrated management

A detailed review by pieterse *et al*. (1992) and parker and Riches (1993) suggesting the possible combination of relevant control methods for Orobanche in a number of susceptible individual crops, still remains very important. However, the following integrated control approach was suggested by Dhanapal *et al*. (2001) for *O.cernua* control in tobacco in India:

- Grow trap crops (sunnhemp/ greengram) in the early spring and incorporate in situ 45 days after sowing.
- Transplant tobacco after 15-20 days.
- Take up general weeding within 45 days after transplanting (DAT).
- Apply glyphosate at 60 DAT at 0.5 kg a.i. ha^{-1} (or less).
- Remove the remaining few broomrapes spikes by hand or apply plant oils to prevent seed formation.

For *o.crenata* control in faba in morocco, the package should include:

a) Treatment with glyphosate;
b) Crop rotation with non-host and avoidance of planting host crops for at least 3-4 years in the same field
c) Hand weeding of the remaining *Orobanche* shoots before and after crop harvest and removal of shoots from the field and burning (Kroschel., 2001).

Three isolates of *Trichoderma* species including *T. harzianum* and *T. viride* were tested for control of Orobanche species in peas, faba bean and tomatoes under field conditions in Egypt. Results of field studies showed that soil treatment with

these three fungal agents alone or soil treatment with fungal agents plus aerial spray of glyphosate (50 ppm) was efficient and cost-effective method in reducing infection, minimizing the number of spikes parasitic on host plants and increasing yields of peas, faba bean and tomatoes (Mokhtar *et al.*, 2009).

CHAPTER THREE

Materials and methods

3.1. General

A series of laboratory experiment was undertaken at the College of Agricultural Studies, Sudan University of Science and Technology (SUST) at Shambat, to **i)** determine the reaction of two safflower varieties to *O.crenata* snd *P.ramosa* and their ability to sustain successful parasitism and **ii)** ability of air dried parts and root exudates of the safflower plants to induce germination of the parasite.

3.2. Materials

3.2.1. Plant materials

Two safflower varieties, Giza and Dongla were obtained from Shambat, College of Agricultural Studies. Sudan University of Science and Technology (SUST).

3.2.2. Seed varieties sterilization

The seeds of safflower varieties were surface-sterilized in 1% 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 bottle till used.

3.2.3. Strigol analogue (GR24) stock solution

A stock solution of the synthetic germination stimulants GR24 (Fig 3.1) 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).

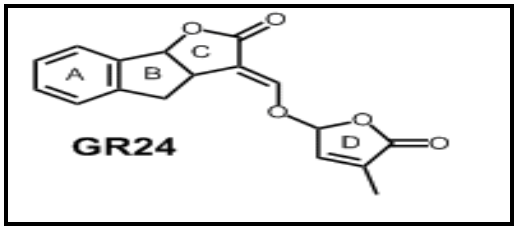

Fig 3.1. Chemical structure of the *Orobanche* germination stimulants GR24

3.2.4. Preparation of Agar medium

Low nutrient agar medium (gelling temperature 30-31ºC, Nacalai Tesque, Kyoto, Japan) was prepared by adding 7.5 g to one liter of distilled water and subsequent autoclaving at 15 bars and 121 ºC for 15 minutes. The autoclaved agar was cooled to room temperature.

3.3. Methods

3.3.1 Laboratory experiments

Laboratory experiments were conducted at the Weeds Research Laboratory, at the College of Agricultural Studies, Sudan University of Science and Technology, to determine ability of residues and root exudates of two safflower varieties (Giza and Dongla) to induce *(O. crenata* and *P. ramosa)* germination, modulate radical extension and haustorium initiation.

3.3.1.1. broomrape seeds sterilization and conditioning

(O. crenata, P. ramosa) seeds were surface sterilized by immersion in 70% ethanol for 2-3 min, followed by washing 3 times with distilled sterilized water. The seeds were then immersed with swirling into a 1% sodium hypochlorite, obtained by appropriate dilution of commercial sodium hypochlorite (Bleach) for 2-3 min, followed by washing with sterilized distilled water to remove all traces of the sterilizing solution. The seeds, plotted on filter papers, were air-dried and stored till used. 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 papers, were moistened with 5 ml of distilled water. Subsequently, about 25-50, surface sterilized *P. ramose* and *O. crenata* 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 20 ºC, for 5 to 7 days.

3.3.1.2. Effects of safflower residues on *O.crenata* and *P.ramosa* seeds germination

The present investigation was undertaken to study the effects of dried safflower varieties residues (leaves, stem, and roots) on ***O.crenata*** and ***P.ramosa*** seed germination, haustorium initiation and radicle length. Micro-multi-well plastic plates were used. Aliquots of autoclaved agar (5ml) as previously described in 3.2.4, were pipette into each well of a multi-well plate. Subsequent to gelatinization samples of safflower parts powder (10, 20, 30, 40 and 50 mg) were added and distributed evenly by hand. Another 5ml Agar was added to each well on top of the sample, and allowed to solidify. This method is known as the sandwich method (Fujii *et al*., 2004). Glass fiber discs containing conditioned *orobanche* seeds (4/well) were placed on top of the second agar layer. Discs containing conditioned *orobanche* seeds, placed on the top of agar, treated with GR24 at 0.1ppm or distilled water, were included as controls for comparison.

The multi-well-plates were sealed with Parafilm, wrapped with aluminum foil and incubated in the dark at 21 ºC for 48 h. The seeds were subsequently examined for germination, radicle length, and haustorium initiation using a binocular stereo-microscope. Treatments were arranged in a Complete Randomized Design (CRD) with 4 replicates.

3.3.1.3. Germination

3.3.1.4. Growth conditions and root exudates collection

Safflower seeds surface sterilized seeds were germinated for 48 h on moistened filter paper at 25°C in darkness. Subsequently, the seedlings were grown hydroponically in 50 mL glass tubes containing 40% Long Ashton (LA) nutrient solution 40 in Biotron (Lighting) at 30°C: 28°C with 16 h: 8 h photoperiod and 70% humidity. The nutrient solution was completed to volume every 24h.The seedlings were removed from the hydroponics system to 4 weeks and the

aqueous phase were collected and extracted with ethylacetate (3x100 ml) (liquid –liquid extraction).

3.3.1.5. Bioassay of broomrape:

Bioassay of crude latex safflower extract:

Aliquots (,10 µl, 15 µl, 20 µl, 25 µl and 30µl) of *Carthamus tinctorius* L latex extract were applied to glass fiber discs and allowed to stand for 2h in a lamina flow to ensure evaporation of ethylacetate. The treated discs were overlaid by discs containing conditioned seeds of the parasites (*O.crenata* and *P.ramosa*) seeds. Each pair of discs was moistened with 40 µl distilled water. The seeds were re-incubated in the dark at 20°C for *O.crenata* and *P.ramosa* Germination was examined after 24 h later for *O.crenata* and *P.ramosa* seeds.

3.4. Statistical analysis:-

Data collected from experiments were subjected to statistical analysis using statistic 8 program statistical for each discs, and subjected to analysis of variance (ANOVA). Means were separated for significance using the least Significant Difference (LSD at $p \geq 0.05$).

CHAPTER FOUR

RESULTS

4.1. Effects of Safflower (root, stem and leaves) powder on *O.crenata* and *P.ramosa*

4.1.1. *O.crenata*

4.1.1.1. Seed germination:

Data presented in (Fig 4.1), showed that Safflower root powder at 10 mg increased germination to 9.7%, while an increase in powder level to 40 and 50 mg increased germination to 9.83 and 11.73% respectively, in Giza. In Dongla at 10, 20 and 30 mg increased germination to 11.46, 16.00 and 14.5%, respectively, nevertheless further increase in level to 40 and 50 mg decreased germination. These reductions were always statistically significante (Table (1) Appendix).

Safflower stem powder at 10, 20 and 40 mg induced germination about 15.66, 11.49 and 14.26% respectively in Giza, while an increase in powder level to 50 mg the germination declined However, 10 and 20mg in Dongla displayed good germination 6 and 7.5% compared with 30, 40 and 50 mg (Fig 4.2).

The results in (Fig 4.3), showed that leaves powder at all levels enhanced germination in both varieties of safflower respectively, while 20 and 50 gm showed maximum germination (51.33 and 50.94%) in Giza. On the other hand, at 10, 20 and 30 mg better germination (60.67, 62.98 and 64.54%) was detected in Dongla respectively.

In general germination of *O.crenata* seeds in response to Safflower, leaves was consistently higher than other parts of the plant powder. The interaction of treatments was significant (Table 4.1.1)

4.1.1.2. Haustorium initiation:

Haustorium initiation was significantly affected by Safflower varieties root powder. Giza showed considerable haustorium initiation. Reduction observed with increase in levels 10 and 20 mg of the powder and gained best haustorium initiation 79.16 and 91.66% in Giza and 75.5 and 80.6% respectively in dongla, albeit this increment was not significant (Fig 4.1, Table (1) Appendix).

Irrespective of Safflower stem powder levels, varieties displayed significant effect on haustoriam initiation (Fig 4.2), levels 30 and 50gm gave maximum haustoriam initiation 91.36 and 91.66%, in Giza. A further increase in stem powder to 40 and 50gm or more appeared to improve haustoriam initiation to 75.33 and 82.6% respectively in Dongla

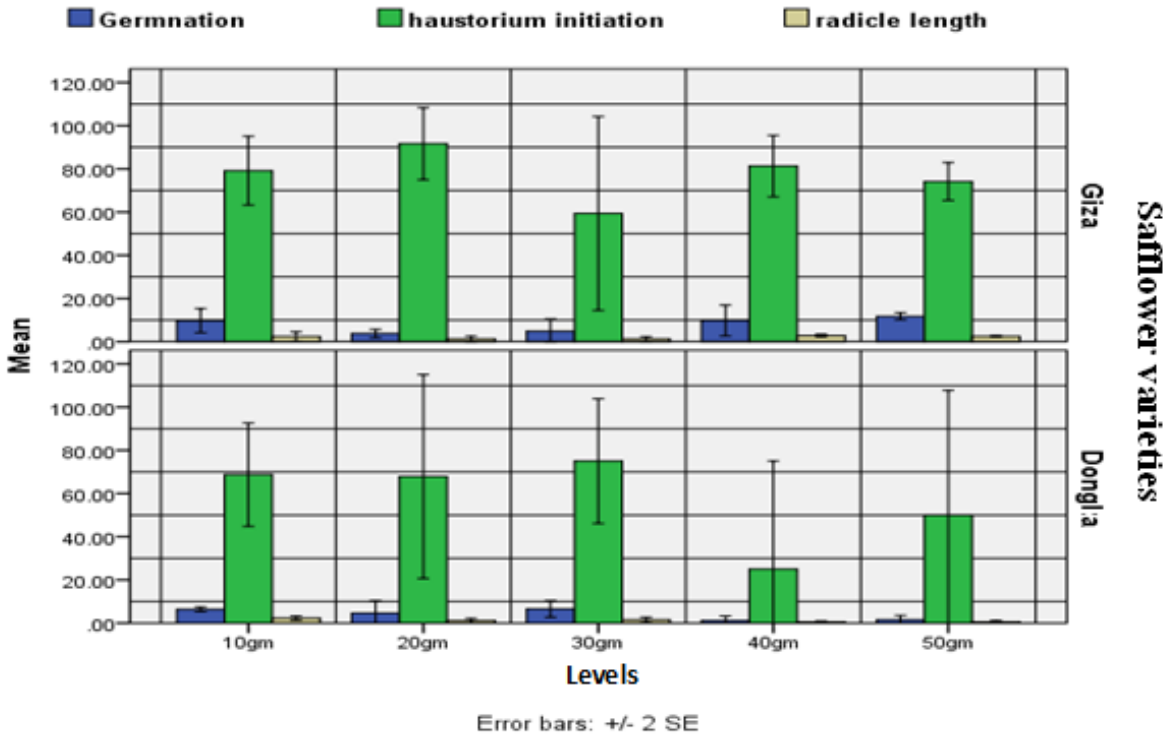

Fig.4.1. Effects of safflower roots powder on O.crenata, germination, haustorium initiation and radicle length

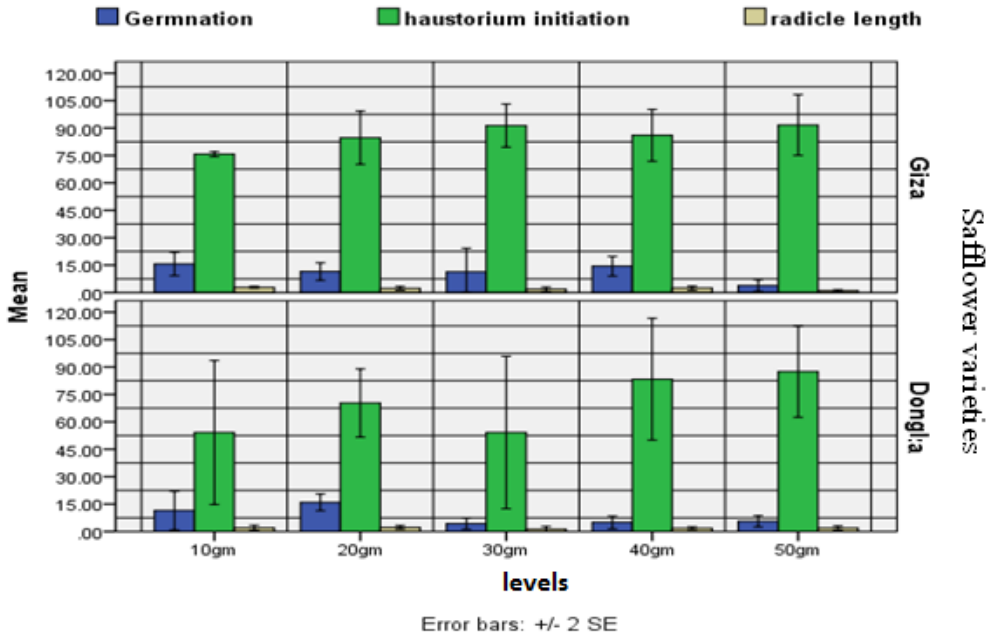

Fig.4.2. Effects of safflower stem powder on O.crenata, germination, haustorium initiation and radicle length

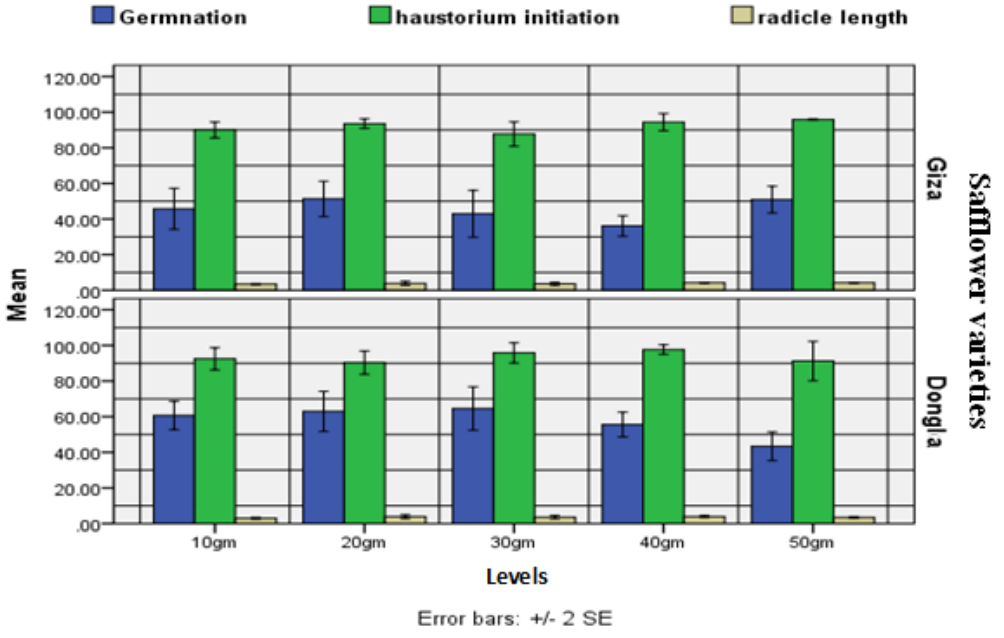

Fig.4.3. Effects of safflower leaves powder on O.crenata, germination, haustorium initiation and radicle length

Data presented in (Fig 4.3), showed that haustorium initiation was neither affected by varieties nor leaves powder levels.

Differences between plant parts powders were not significant. However, increasing stem powder to 50 mg induced slightly more haustoriam followed by root powder.

4.1.1.3. Radical length:

Significant differences were found between varieties in root powder, high radical length was obtained from Giza 2.8 $\mu m10^{-2}$, whereas, Dongla recorded 2.6 $\mu m10^{-2}$ (Fig 4.1). On the other hand, treatments interaction showed statistically different results (table (1) Appendix).

The result in (Fig 4.2and Fig 4.3), showed that both stem and leaves powder had no effect on this parameter. However at 10 and 20mg Giza achieved 2.8 and 2.3 $\mu m10^{-2}$, while dongle achieved 2.3 and 1.12 $\mu m10^{-2}$ respectively.

Table (4.1.1) Effect of varieties and leaves powder level interaction on germination, haustorium and radical length in *O.crenata*

source	Levels	*O.crenata*		
		G	H	R
V1	GR24	61.21a	71.22bc	3.5b
	10 mg	45.74 bc	89.99 ab	3.43 ab
	20 mg	51.33 ab	93.55 ab	3.87 ab
	30 mg	42.93 bc	87.73 b	3.62 ab
	40 mg	36.14 c	94.37 ab	4.18 a
	50 mg	50.94 ab	95.86 ab	4.18 a
V2	10 mg	60.99 a	92.50 ab	3.00 b
	20 mg	62.98 a	90.35 ab	3.87 ab
	30 mg	64.54 a	95.83 ab	3.56 ab
	40 mg	55.57 ab	97.67 a	3.93 ab
	50 mg	43.37 bc	91.23 ab	3.43 ab
LSD	//	14.64	8.79	1.00

v1=Giza, v2=Dongla, G=Germination, H=haustorium, R=radical length, MG= milligram,

[1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

Table (4.1.2) Effect of two safflower varieties and root powder interaction on germination, haustorium and radical length in *O.crenata*

source	Levels	*O.crenata*		
		G	H	R
V1	GR24	54.11a	62.00ab	3a
	10 mg	9.70 ab	79.16 a	2.37 ab
	20 mg	3.78 c	91.68 a	1.25 abc
	30 mg	4.92 bc	59.37 ab	1.18 abc
	40 mg	9.83 ab	81.25 a	2.81 a
	50 mg	11.73 a	74.16 ab	2.50 ab
V2	10 mg	6.33 abc	68.75 ab	2.31 ab
	20 mg	4.62 bc	67.85 ab	1.12 bc
	30 mg	6.54 abc	75.00 ab	1.50 abc
	40 mg	1.08 c	25.00 b	0.43 c
	50 mg	1.54 c	50.00 ab	0.50 c
LSD	//	5.67	52.23	1.62

v1=Giza, v2=Dongla, G=Germination, H=haustorium, R=radical length, MG= milligram,

[1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

Table (4.1.3) Effect of two safflower varieties and stem powder interaction on germination, haustorium and radical length in *O.crenata*

source	Levels	*O.crenata*		
		G	H	R
V1	GR24	25.3a	50.1b	2.50a
	10 mg	15.95 a	75.69 ab	2.87 a
	20 mg	11.49abc	84.74 ab	2.25 abc
	30 mg	11.33abc	91.37 ab	1.81 abc
	40 mg	14.41 ab	86.01 ab	2.50 ab
	50 mg	3.89 c	91.68 a	1.00 c
V2	10 mg	11.46abc	54.16 b	2.00 abc
	20mg	15.95 a	70.29 ab	2.25 abc
	30 mg	4.28 c	54.16 b	1.25 bc
	40 mg	5.00 bc	83.33 ab	1.18 abc
	50 mg	5.60 bc	87.50 ab	4.87 abc
LSD	//	9.60	37.32	1.45

v1=Giza, v2=Dongla, G=Germination, H=haustorium, R=radical length, MG= milligram,

[1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

4.1.2. *P.ramosa*

4.1.2.1. Seed germination:

Root powder of Safflower varieties displayed highly significant effect on germination of *p.rmosa* in (Fig 4.4). Seed treated with 30 mg on Giza gained considerable germination 61.22%. Similar rate was found in dongla which obtained 45%. A further increase in powder level resulted in significant decline in germination (table (1) Appendix).

Safflower stem powder induced germination in Giza. An increase in powder level to 50mg showed high germination (30.57%), more than in dongla which was 28.2% at the same level, compared with other levels (Fig 4.4).

The highest seed germination of *P.ramosa* was observed when seed treated with dongla leaves powder (Fig 4.3). It appeared that 20 and 40gm gained better germination 56 and 55% than other levels. Giza showed high germination with 30 and 50 gm in comparison with other levels.

4.1.2.2. Haustorium initiation:

Deference between varieties was not occurred when seed treated by root powder at all levels. Moreover, the interaction of factors was not significant. Nevertheless more haustorium formation detected in dongla at high levels (Fig 4.4).

Haustorium initiation was highly significant when used stem powder. Deference between varieties was occurred (Fig 4.5), Giza stem powder at 10 and 50mg. induced considerable number of haustoria 77. 62 and 62.5% respectively. While dongla at 10 and 50 mg slightly induced 35.5 and 39.16% haustoria initiation respectively. An increase in stem powder to 30 and 40mg decreased haustorium formation to 17 and 27.16% respectively (Fig 4.5).

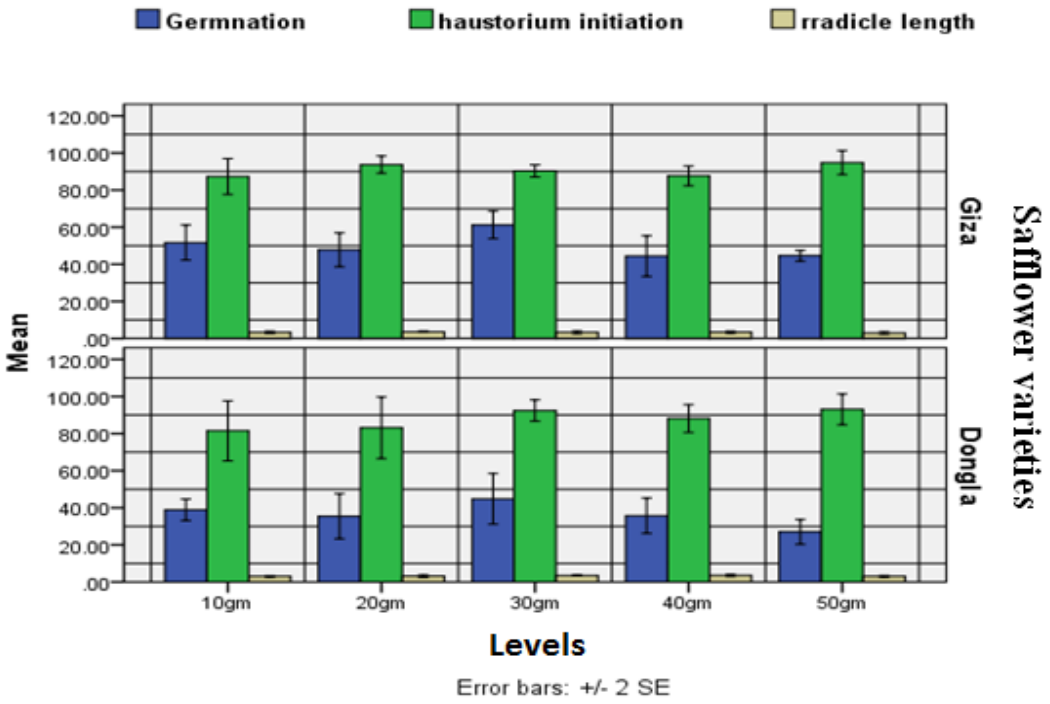

Fig.4.4. Effects of safflower roots powder on P.ramosa , germination, haustorium initiation and radicle length

Irrespective of leaves powder levels, Giza leaves powder at 30 mg induced 100% haustorium initiation, while at 10 and 50gm recorded about 97 and 92.3% respectively in Dongle (Fig 4.6)

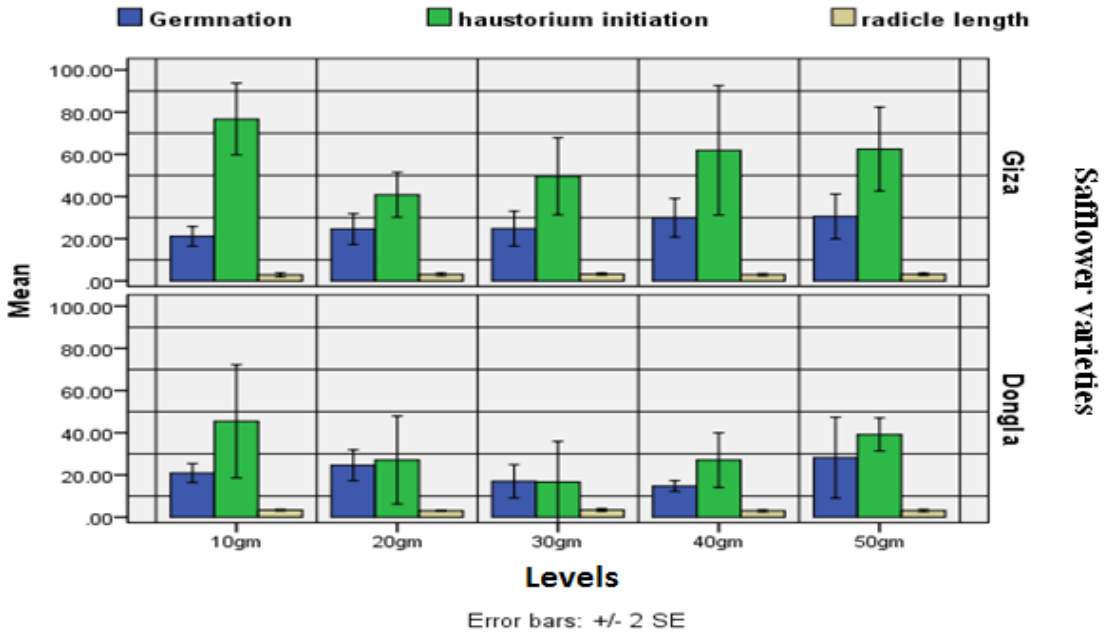

Fig.4.5. Effects of safflower stem powder on P.ramosa, germination, haustorium initiation and radicle length

4.1.2.3. Radical length:

Data in (Fig 4.6), showed that Safflower leaves powder produced significant radical length at all levels in comparison to other part of the plant. At level 40gm Dongla recorded 3.6 $\mu m10^{-2}$ which was the best one.

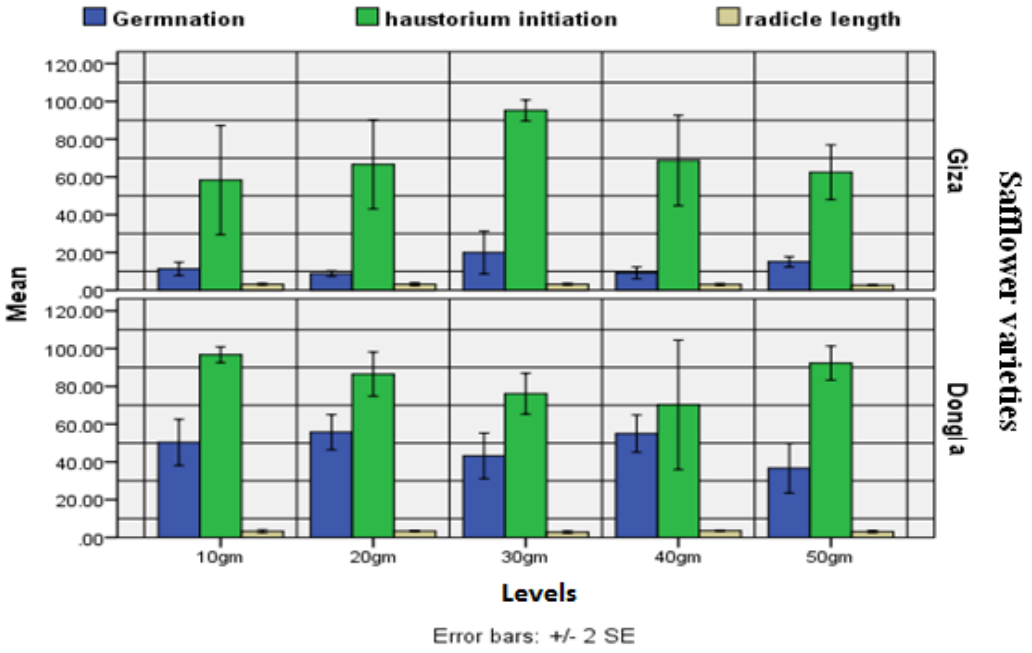

Fig.4.6. Effects of safflower leaves powder on P.ramosa, germination, haustorium initiation and radicle length

Table (4.1.4) Effect of two safflower varieties and leaves powder interaction on germination, haustorium and radical length in *O.crenata*

Source	Levels	*P.ramosa*		
		G	H	R
V1	GR24	61.22a	80.32ab	2.20b
	10 mg	11.32 c	58.32 e	3.18 ab
	20 mg	8.82 c	66.66 cde	3.18 ab
	30 mg	20.02 c	95.26 abc	3.18 ab
	40 mg	9.19 c	68.7 bcde	3.06 ab
	50 mg	15.11 c	62.50 de	2.75 b
V2	10 mg	50.34 a	96.74 a	3.25 ab
	20 mg	55.73 a	86.47 abcd	3.37 ab
	30 mg	43.23 ab	76.07 abcde	2.81 ab
	40 mg	55.00 a	70.20 abcde	3.56 a
	50 mg	36.71 b	92.26 abc	3.06 ab
LSD	//	13.56	27.52	0.79

v1=Giza, v2=Dongla, G=Germination, H=haustorium, R=radical length, MG= milligram,

[1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

Table (4.1.5) Effect of two safflower varieties and root powder interaction on germination, haustorium and radical length in *P.ramosa*

Source	Levels	*P.ramosa*		
		G	H	R
V1	GR24	60.00a	80.22a	3.00a
	10 mg	51.70 ab	87.15 a	3.25 a
	20 mg	47.81abc	93.74 a	3.62 a
	30 mg	61.23 a	90.34 a	3.25 a
	40 mg	44.46 bc	87.29 a	3.37 a
	50 mg	44.67 bc	94.81 a	3.00 a
V2	10 mg	38.93bcd	81.47 a	3.00 a
	20 mg	35.45 cd	83.14 a	3.12 a
	30 mg	44.85 bc	92.42 a	3.50 a
	40 mg	35.75 cd	88.10 a	3.56 a
	50 mg	27.03 d	93.05 a	3.00 a
LSD	//	13.52	14.03	0.84

v1=Giza, v2=Dongla, G=Germination, H=haustorium, R=radical length, MG= milligram,

[1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

Table (4.1.6) Effect of two safflower varieties and stem powder interaction on germination, haustorium and radical length in *P.ramosa*

source	Levels	*P.ramosa*		
		G	H	R
V1	GR24	40.00a	80.22a	3.00a
	10 mg	21.12 abc	76.73 a	2.88 a
	20 mg	24.61 abc	40.83 bcd	3.06 a
	30 mg	24.81 abc	49.58 abc	3.25 a
	40 mg	29.92 ab	61.87 ab	2.93 a
	50 mg	30.58 a	62.50 ab	3.12 a
V2	10 mg	20..89 abc	45.44 bcd	3..37 a
	20 mg	24.61 abc	27.08 cd	3.06 a
	30 mg	16.99 bc	16.66 d	3.43 a
	40 mg	14.76 c	27.80 cd	3.00 a
	50 mg	28.22 ab	39.16 bcd	3.12 a
LSD	//	13.44	29.72	0.84

v1=Giza, v2=Dongla, G=Germination, H=haustorium, R=radical length, MG= milligram,

[1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

4.2. Effect of Safflower root exudates on broomrape seed germination, haustorium and radical length

4.2.1. First week:

Analysis of data, showed that the effect of safflower root exudates on haustorium and radical length of *O.crenata* was significant however, the effect on seed germination was not significant (Table 4.2.1). Giza root exudates achieved high effect 5**.**70 and 2.72% on haustorium and radical length respectively, compared to Dongla which were 0.00 and 2.08% at the first week of germination (table 4.2.3). *O.crenata* seed germinate with GR24 and level 1 (10µl) were 52.9 and 40.4% respectively. Root exudates at the lower level (10µl) increased germination, haustorium initiation and radical length. Increasing levels to 3 and 4 (20-25µl) elicited all parameters (Table 4.2.3). On the other hand, no haustoria were initiated in *P.ramosa* germblings treated with root exudates of the two varieties of safflower. However, irrespective of varieties, root exudates level displayed high significant difference ($p \leq 0.01$) on radical length (Table 4.2.1). Giza achieved high values 36.39% compared to Dongla 34.30%.

Interaction between varieties and level displayed high significant on all parameters of *O.crenata* ($p \leq 0.01$). However it appeared that high significant on germination, radical length and was not significant on houstorum initiation of *P.ramosa* (Table 4.2.1), (Table 4.2.2).

Table (4.2.1): Analysis of variance (ANOVA) summary effects of treatments on germination , haustorium and radical length of broomrape at first week

Source of variation	*O.crenata*				*P.ramosa*			
	DF	G	H	R	DF	G	H	R
Sv	1	0.55^{ns}	59.96^{**}	12.45^{**}	1	0.12^{ns}	M^0	6.09^{*}
Level	5	9.75^{**}	30.5^{**}	5.17^{**}	5	11.82^{**}	M^0	10.88^{**}
Sv*level	5	5.87^{**}	30.50^{**}	5.81^{**}	5	12.7^{**}	M^0	9.61^{**}
Error	33				33			

*=P<0.05, **=P<0.01, ^{NS} = not significantly different at P = 0.05. Key: Sv = safflower verities G= germination H= haustorium R= radical length

Table (4.2.2) Effect of varieties and parasites on germination, haustorium and radical length one week after germination

source	Levels	*O.crenata*			*P.ramosa*		
		G	H	R	G	H	R
V1	GR24	60.45 a	0.00 c	4.31 a	48.70 a	0.00 c	4.75 a
	10µl	51.77ab	24.37a	3.62 a	10.32def	0.00 c	2.31 bc
	15µl	9.20 e	0.00 c	1.68 b	2.22 f	0.00 c	0.56 d
	20µl	35.00cd	9.82 b	1.93 b	14.97 cd	0.00 c	2.87 b
	25µl	26.02 d	0.00 c	2.25b	11.80 def	0..00 c	2.31 bc
	30µl	35.87cd	0.00 c	2.50 b	13.30cde	0.00 c	2.56 b
V2	GR24	45.27bc	0.00 c	2.05 b	18.100cd	0.00 c	3.12 b
	10µl	28.92 d	0.00 c	1.90 b	32.17 b	0.00c	1.31 b
	15µl	33.5cd	0.00 c	2.05 b	15.30 cd	0.00 c	2.87 b
	20µl	31.87cd	0.00 c	2.00 b	3.00 ef	0.00 c	0.25 d
	25µl	31.05 d	0.00 c	2.00 b	24.05 bc	0.00 c	2.56 b
	30µl	34.77cd	0.00 c	2.45 b	13.37cde	0.00 c	2.18 bc
LSD	//	13.92	3.67	0.90	11.01	7.50	1.09

V1= Giza, V2= Dongla, G= germination H= houstorum R= radical length µl= micro liter,[1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

Table (4.2.3): Effects of two safflower varieties and root exudates on germination, haustorium and radical length of broomrape at first week

Source	*O.Crenata*			*P.ramosa*		
	G	H	R	G	H	R
V1	36.39 a	5.70 a	2.72 a	16.89 a	0.00 c	2.56 a
V2	34.31 a	0.00 c	2.08 b	17.67 a	0.00 c	2.02 b
LSD	5.69	1.50	0.37	4.50	0.00 c	0.44
GR24	52.86 a	0.00 c	3.18 a	33.40 a	0.00 c	3.94 a
Level 1	40.35 b	12.19 a	2.76 ab	21.25 b	0.00 c	1.78 bcd
Level 2	21.57 d	0.00 c	1.87 c	8.76 d	0.00 c	1.66 cd
Level 3	33.44 c	4.91 b	1.97 c	8.99 d	0.00 c	1.56 d
Level 4	28.54 cd	0.00 c	2.12 bc	17.93 bc	0.00 c	2.44 b
Level 5	35.33 bc	0.00 c	2.47 bc	13.34 cd	0.00 c	2.37 bc
LSD	9.85	2.59	0.64	7.79	0.00 c	0.77

Means in the same column with same letter are not significantly different. Key: V1= Giza, V2= Dongle, level1=10 µl, level2=15 µl, level3=20 µl, level4=25 µl, level5=30 µl.G= germination H= haustorium R= radical length

4.2.2. Second week:

No variation between varieties observed at two weeks after germination, on haustorium initiation and radical length of *O.crenata* seed (Table 4.2.4) Nevertheless, all parameters responded to exudates levels consistently higher significant. Increasing level to level 6 (30µl) and level 5 (25µl) achieved high values on germination, haustorium initiation and radical length (28, 30.8, 51.2% and 5.5 µm10^{-2}) respectively (table 4.2.4).

P.ramosa grembling seeds treated by exudates showed high significant on haustorium and radical length (Table 4.2.4). No haustorium initiated in Dongla where as Giza achieved 12.89% (Table 4.2.6).

Table (4.2.4): Analysis of variance (ANOVA) summary effects of treatments on germination, haustorium and radical length of broomrape at second week

Source of variation	*O.Crenata*				*P.ramosa*			
	DF	**G**	**H**	**R**	**DF**	**G**	**H**	**R**
Sv	1	0.24^{ns}	0.12^{ns}	0.08^{ns}	1	2.76^{ns}	41.29^{**}	36.66^{**}
Level	5	8.55^{**}	24.37^{**}	11.61^{**}	5	7.73^{**}	11.44^{**}	3.01^{*}
Sv*level	5	0.27^{ns}	3.75^{**}	0.07^{ns}	5	1.25^{ns}	11.44^{**}	3.31^{*}
Error	33				33			

*=P<0.05, **=P<0.01, ^{NS} = not significantly different at P = 0.05. Key: Sv = safflower verities G= germination H= haustorium R= radical length.

Root exudates level showed high significant germination, haustorium initiation, and radical length of ramosa ($p \leq 0.01$) (table 4.2.4). level4 (25µl) had high effect on germination and haustorium whereas level3 (20µl) on radical length (16.06, 16.88 and 4.50 $\mu m10^{-2}$) respectively (table 4.2.6)

Interaction of varieties and levels showed not significant on all parameters of broomrape except haustorium initiation and radical length ($p \leq 0.01$). (table 4.2.5).

Table (4.2.5) Effect of varieties and broomrape on germination, haustorium and radical length one week after germination

source	Levels	*O.crenata*			*P.ramosa*		
		G	H	R	G	H	R
V1	GR24	51.35 a	0.00 f	6.06 a	27.17 a	4.16 b	4.56 bc
	10µl	21.50 b	20.31cde	3.50 bc	17.05 bc	0.00 c	4.00 bcd
	15µl	21.12 b	0.00 f	3.75 bc	15.52 bc	0.00 c	4.75 bc
	20µl	24.75 b	0.00 f	3.25 c	15.52 bc	34.58 a	6.56 a
	25µl	21.02 b	31.55 bc	5.50 a	13.52 bc	33.75 a	5.56 ab
	30µl	27.97 b	51.20 a	4.87 a	18.07 b	4.79 b	3.25 cde
V2	GR4	51.35 a	0.00 f	6.06 a	27.17 a	0.00 c	4.56 bc
	10µl	22.42 b	6.47 ef	3.25 c	8.72 c	0.00 c	2.25 e
	15µl	20.85 b	20.00cde	3.87 bc	8.60 bc	0.00 c	2.18 e
	20µl	24.75 b	14.93 de	2.87 c	9.95 ab	0.00 c	2.43 de
	25µl	30.77 b	22.84 cd	5.50 a	18.60 ab	0.00 c	2.50 de
	30µl	27.45 b	44.83 ab	4.87 ab	13.00 bc	0.00 c	2.43 de
LSD	//	15.73	39.7	1.45	8.71	9.99	1.68

V1= Giza, V2= Dongla, G= germination H= houstorum R= radical length µl= micro liter,[1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

Table (4.2.6): Effects of two safflower varieties and root exudates on germination, haustorium and radical length of broomrape at second week

Source	*O.Crenata*			*P.ramosa*		
	G	H	R	G	H	R
V1	28.05 a	17.18 a	4.49 a	17.25 a	12.88 a	4.78 a
V2	29.60 a	18.18 a	4.40 a	14.34 a	0.00 c	2.73 b
LSD	6.43	5.83	0.59	3.56	4.07	0.68
GR24	51.35 a	0.00 d	6.06 a	27.18 a	2.08 b	4.56 a
Level 1	21.97 b	13.39 b	3.37 c	12.89 b	0.00 b	3.84 b
Level 2	20.99 b	10.00 cd	3.81 c	10.26 b	0.00 b	3.47 ab
Level 3	25.02 b	7.47 cd	3.06 c	12.85 b	17.29 a	4.50 a
Level 4	25.90 b	27.20 b	5.50 ab	16.06 b	16.88 a	4.03 ab
Level 5	27.71 b	48.02 a	4.87 b	15.54 b	2.39 b	2.84 b
LSD	11.13	10.11	1.02	6.16	7.06	1.19

Means in the same column with same letter are not significantly different. Key: V1= Giza, V2= Dongle, level1=10 µl, level2=15 µl, level3=20 µl, level4=25 µl, level5=30 µl.G= germination H= haustorium R= radical length

4.2.3. Third week:

According to statistical analysis, it was obvious that the effect of safflower varieties exudates on germination, houstorum initiation and radical length of *O.crenata* seed was significant at 21 days after germination (table 4.2.7). Giza achieved high values (27.44%) compared to Dongla (17.15%) (Table 4.2.9).

Table (4.2.7): Analysis of variance (ANOVA) summary effects of treatments on germination , haustorium and radical length of broomrape at third week

Source of variation	*O.Crenata*				*P.ramosa*			
	DF	G	H	R	DF	G	H	R
Sv	1	12.85^{**}	6.29^{*}	30.27^{***}	1	4.11^{*}	4.73^{*}	14.03^{***}
Level	5	2.85^{*}	1.35^{ns}	0.66^{ns}	5	6.04^{***}	1.59^{ns}	4.13^{***}
Sv*level	5	4.62^{**}	1.22^{ns}	3.89^{**}	5	3.66^{**}	1.86^{ns}	13.90^{***}
Error	33				33			

*=P<0.05, **=P<0.01, ***=P<0.001, NS = not significantly different at P = 0.05. Key: Sv = safflower verities G= germination H= haustorium R= radical length

Root exudates levels showed significant effect on germination, of *O.crenata* seed and was not significant on haustorium initiation, and radical length (table 4.2.7). Application of 10, 15 and 20µl increased germination by 26.60, 26.81 and 26.21% respectively (Table 4.2.9).

Interaction between factors displayed high significant on germination, and radical length of *O.crenata* seed and was not significant on haustorium initiation ($p \leq 0.01$) (table 4.2.7). Moreover, root exudates levels showed high significant on seed germination, radical length and was not significant in haustorium initiation of *P.ramosa* (table 4.2.7). Besides 25 µl produced more germination than others. Increasing level increased radical length, whereas 15µl had significant haustorium initiation (table 4.2.9). Interaction between varieties and levels gained high significant on germination and radical length ($p \leq 0.01$) of *P. ramose* and *O.crenata* (Table 4.2.8).

Table (4.2.8) Effect of varieties and broomrape on germination, haustorium and radical length at three week after germination

source	Levels	*O.crenata*			*P.ramosa*		
		G	H	R	G	H	R
V1	GR24	23.45 bc	0.00 b	3.75 cd	27.17 a	0.74 c	27.18 a
	10µl	43.40 a	0.00 b	4.37 bc	11.50 bc	2.74 c	4.56 a
	15µl	31.57 ab	0.00 b	4.12 bcd	10.30de	0.00 c	2.31 bc
	20µl	37.85 a	1.66 b	5.00 ab	9.75 cd	0.40 c	2.93 b
	25µl	18.22 bc	0.00 b	4.62 abc	17.42 bc	0.00 c	2.87 b
	30µl	10.12 c	0.00 b	5.44 a	16.07 bc	0.00 c	3.00 b
V2	GR4	23.50 bc	0.00 b	3.75 cd	14.45 bc	0.00 c	2.93 b
	10µl	9.80 c	13.12 a	3.31 de	3.92 e	0.00 c	0.50 d
	15µl	22.05 bc	2.00 b	3.68 cd	14.77 bc	30.29 a	4.68 a
	20µl	14.57 bc	7.22 ab	3.62 cd	13.80 bc	5.00 bc	2.25 bc
	25µl	19.27 bc	8.36 ab	3.62 cd	19.45 ab	0.00 c	3.06 b
	30µl	13.72 c	0.00 b	2.56 e	5.67 de	25.00 ab	1.56 cd
LSD	//	14.30	9.61	1.01	10.40	21.54	1.16

V1= Giza, V2= Dongla, G= germination H= houstorum R= radical length µl= micro liter,[1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

Table (4.2.9): Effects of two safflower varieties and root exudates on germination, haustorium and radical length of broomrape at third week

Source	*O.Crenata*			*P.ramosa*		
	G	H	R	G	H	R
V1	27.44 a	0.28 b	4.55 a	15.37 a	0.65 b	3.37 a
V2	17.15 b	5.12 a	3.43 b	12.01 a	10.05 a	2.50 b
LSD	5.84	3.93	0.62	2.03	8.80	0.47
GR24	23.48 a	0.00 c	3.75 a	20.81 a	0.37 a	3.75 a
Level 1	26.60 a	6.56 a	3.84 a	7.71 b	1.37 a	2.53 b
Level 2	26.81 a	1.00 a	3.90 a	12.54 b	15.15 a	3.50 a
Level 3	26.21 a	4.44 a	4.31 a	11.77 b	2.70 a	2.53 b
Level 4	18.75 ab	4.18 a	4.13 a	18.44 a	0.00 a	2.97 ab
Level 5	11.93 b	0.00 a	4.00 a	10.87 b	12.50 a	2.28 b
LSD	5.84	6.80	0.72	2.03	15.23	0.82

Means in the same column with same letter are not significantly different. Key: V1= Giza, V2= Dongle, level1=10 µl, level2=15 µl, level3=20 µl, level4=25 µl, level5=30 µl.G= germination H= haustorium R= radical length

4.2.4. Four week

Differences between varieties was significant on radical length of *o.crenata* (Table 4.2.10). Dongle achieved high effect compared to Giza in radical length after 28 days of germination (Table 4.2.12). Root exudates levels was highly significant on germination and high on radical length ($p \leq 0.01$) (table 4.2.10). Seed treated with 15µl exudates levels, recorded high value (20.05%) of germination. Also 15 and 20µl levels showed considerable radical length. On the other hand, seed germination and haustorium initiation of *P.ramosa* responded significantly to varieties root exudates (table 4.2.10). Giza achieved high values on germination (12.63 %) compared to Dongla which was (9.08%). However, Dongla recorded high value of radical length 18.40 $\mu m10^{-2}$ compared to Giza 2.78 $\mu m10^{-2}$ (Table 4.2.12).

Table (4.2.10): Analysis of variance (ANOVA) summary effects of treatments on germination , haustorium and radical length of broomrape at four week

Source of variation	*O.Crenata*				*P.Ramosa*			
	DF	G	H	R	DF	G	H	R
Sv	1	0.36^{ns}	0.00^{ns}	5.99^{*}	1	5.57^{*}	49.04^{**}	1.33^{ns}
Level	5	15.90^{**}	1.38^{ns}	3.51^{*}	5	12.10^{**}	50.71^{**}	8.83^{**}
Sv*level	5	1.48^{ns}	1.57^{ns}	0.51^{ns}	5	0.76^{ns}	58.46^{**}	0.13^{ns}
Error	33				33			

*=P<0.05, **=P<0.01, ^{NS} = not significantly different at P = 0.05. Key: Sv = safflower verities G= germination H= haustorium R= radical length

Root exudates levels showed high significant on *P.ramose* seed germination, haustorium and radical length (table 4.2.10). GR24 had high effect on germination, while levels 10 to 30 µl was significant on germination (Table 4.2.12).

Interaction between varieties and level appeared significant on, haustorium initiation only ($p \leq 0.01$) (table 4.2.11).

Table (4.2.11) Effect of varieties and broomrape on germination, haustorium and radical length at four week after germination

Source	Levels	*O.crenata*			*P.ramosa*		
		G	H	R	G	H	R
V1	GR24	31.67 a	30.55 a	2.56 bc	23.85 a	4.16 bc	4.25 a
	10µl	8.97 ef	6.25 b	2.37 bc	12.15 b	0.00 c	2.25 b
	15µl	22.90 bc	2.27 b	2.93 abc	7.42 bc	0..00 c	1.75 b
	20µl	10.87 def	0.00 b	2.56 bc	11.87 b	12.50 c	2.50 b
	25µl	9.67 def	0.00 b	2.00 c	8.60 bc	0.00 c	2.25 b
	30µl	11.52 def	0.00 b	1.93 c	11.67 b	30.00	1.81 b
V2	GR4	25.05 ab	3.12 b	3.31 ab	23.85 a	4.16 bc	4.25 a
	10µl	7.10 f	16.66 ab	2.68 abc	3.97 c	6.25 bc	1.75 b
	15µl	17.20 cd	10.60 ab	3.43 ab	7.42 bc	0.00 c	2.43 b
	20µl	15.10 de	8.58 ab	3.75 a	6.95 bc	0.00 c	1.87 b
	25µl	12.40 def	0.00 b	2.43 bc	6.07 bc	100.00 a	1.87 b
	30µl	13.30 def	0.00 b	2.00 c	6.17 bc	0.00 c	1.43 b
LSD	//	7.60	22.98	1.10	7.50	11.11	1.39

V1= Giza, V2= Dongla, G= germination H= haustorium R= radical length µl= micro liter, [1]Means within a row followed by the same letter(s) are not significantly different at P≤ 0.5

Table (4.2.12): Effects of two safflower varieties and root exudates on germination, haustorium and radical length of broomrape at four week

Source	*O.Crenata*			*P.ramosa*		
	G	H	R	G	H	R
V1	15.94 a	6.51 a	2.39 b	12.63 a	2.78 b	2.47 a
V2	15.03 a	6.50 a	2.94 a	9.08 b	18.40 a	2.15 a
LSD	3.10	9.38	0.45	3.06	4.54	0.57
GR24	28.36 a	16.84 a	2.94 ab	23.85	4.17 b	4.25 a
Level 1	8.04 c	11.46 b	2.53 abc	8.06 b	3.13 b	2.00 bc
Level 2	20.05 b	6.44 b	3.19 a	7.52	0.00 c	1.44 c
Level 3	12.99 c	4.29 b	3.16 a	9.41 b	6.25 b	2.47 b
Level 4	11.04 c	0.00 c	2.22 bc	7.34 b	50.00 a	2.06 bc
Level 5	12.41 c	0.00 c	1.97 c	9.41 b	0.00 c	1.62 bc
LSD	5.38	16.25	0.78	5.30	7.86	0.98

Means in the same column with same letter are not significantly different. Key: V1= Giza, V2= Dongle, level1=10 µl, level2=15 µl, level3=20 µl, level4=25 µl, level5=30 µl.G= germination H= haustorium R= radical length

CHAPTER FIVE

Discussion

This study described the reaction of two safflower varieties powder and exudates on germination, haustorium and radical length of two broomrape (*O. crenata* and *P.ramosa*) in Petri dishes. Petri dishes experiments detailed investigations on the capacity of safflower plant to induce broomrape seed germination and their ability to limit attachment and development of the parasite. This method is faster and cheaper than the field experiments and allows homogenization of orabanche seed distribution. The interactions of the different plant species with broomrape can be classified into three distinct categories.

Host plants stimulate parasitic seed germination, tubercle development and seed production; false-host plants stimulate parasitic seed germination without tubercle formation; and non-host plants stimulate neither parasitic seed germination nor attachment Host plant species release exudates that stimulate the germination and the attachment of parasitic seeds, while false-host species release exudates that only stimulate germination (Joel, 1995). Thus, an additional chemical signal is needed for the radicle to penetrate host roots and to form a haustorium serving as a bridge for water and nutrient uptake from the host to the parasite. Since false-hosts can stimulate parasitic seed germination without attachment, they could be used as trap crops to reduce the amount of parasitic seeds in infested soil.

In this study, *O. crenata* seems to be more pathogenic on faba bean than on other legumes and *P.ramosa* on tomato. The results of laboratory experiment showed that safflower residues, irrespective of varieties, plant parts, amount of powder and exudates, induced germination of broomrape (*O. crenata* and *P.ramosa*) . Exudates of safflower roots induced Orobanche germination, through their

ability to do so was good as GR24, therefore, the quantity or activity of stimulant produced was activated germination, haustorium formation, and subsequent development.

In general germination of (*O. crenata* and *P.ramosa*) seeds were maximal at higher level of extract and/ or powder. More over the exudates displayed higher activity than powder form, The higher activity of extract may be attribute to solubilization of the active material in the exudates component. This finding is in the line with Tilal sayed (2012) who studied the effect of *Euphorbia hirta* extract on *P.ramosa* seed germination.

Irrespective of varieties, leaves powder induced germination of *O.crenata* seeds consistently higher than other parts of the plant.

Between two safflower varieties Giza root and stem powder gave the highest germination in *P.ramosa* respectively. Leav powder induced germination in Dongla variety. The difference between varieties may be related to differential stimulants contents of the respective powders or differential sensitivity of the parasite.

The experiment demonstrated that root and stem powder of safflower when applied to germbling seed of *O.crenata* induced haustorium formation significantly Increasing stem powder to 50 mg initiated more haustorium with two varieties. It was clear that haustorium intiation in P.*ramose* affected by stem and leaf powder. An increased in stem powder to 40 and 50 gm decreased haustorium formation. Giza leaves powder at 30 mg induced 100% haustorium initiation.

In general the observed differences in seedlings mortality may be attributed to difference in the ability of the hosts to supply nutrients to the parasite. A similar phenomenon was reported for Framida, on Striga resistant sorghum variety

(Arnand *et al*., 1991, Amusan *et al*., 2008) and was attributed to blockage of translocation of assimilates and/or other host metabolites essential for growth and development of the parasite, thus leading to haustorial collapse and/or inhibition of the haustorial development culminating in death and/or slow growth of the parasite. Root exudates of Giza achieved high effect 5**.**70 and 2.72% on haustorum and radical length respectively compared to dongla which were 0.00 and 2.08% at the first week of germination in crenata . According to statistical analysis it was obvious that the effect of safflower varieties exudates on germination, houstorum initiation and radical length of *O.crenata* seed was significant at three weeks after germination. Giza achieved high values (27.44%) compared to Dongle (17.15%) On the other hand, no houstoria were initiated in *P.ramosa* germblings treated with root exudates of varieties. Furthermore, at four week after germination root exudates displayed highly significant effect on haustorium initiation in *P.ramosa* Root powder enhanced redical elongation in O.*crenata* ,whereas leaf powder in *P.ramose* gained high length of redical.

The concurrent differential increase or decrease in germination and haustorium initiation with increasing powder levels may be attributed to difference in the chemical signals involved and the cytological changes in the radical. It is believed that germination of parasitic weed is affected by join action of germination stimulants and ethylene produced by the seed (Babiker *et al*., 2000).

Reports on induction of *Phelipanche* and *Orobanche spp* germination by ethylene controversial and early work did not supports involvement of ethylene in *P ramose* germination(Parker and Riches, 1993). However, later reports By Zehher *et al*., (2002) indicated that possible involvement of ethylene in *P. ramosa* germination.

The active compound in safflower should identify as they may offer or lead to template for synthesize of more active and stable compound that induce and /or

inhabit germination and haustorium intiation. In general, search for seed germinatiom in plant residues should be receive more attention as plant residues can be play an important role in weed control and soil protection.

Conclusions and Recommendations

Conclusions

- Safflower is susceptible to *O.crenata P.ramosa* and was able to support the germination, attachment and subsequent of broomrape.
- Safflower residues and root exudates induced germination, haustorioum initiation and reduced radical length of *O*. *crenata* and *P*. *ramosa*

Recommendations

- Further research is needed to find out whether differences exist in the onset of stimulant exudation by the roots of safflower cultivars.
- More elaborate studies on the influence of safflower varieties on broomrape are needed in the field. Employment of safflower as a trap crop for O. *crenata,* a serious pest on faba bean (*Vicia faba* L.) and *P*. *ramosa* on tomato (*Lycopersiction esculentum* Mill) in the Northern and River Nile States should be investigated.

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Appendices

Appendix 1

Table (1): Effects of safflower root powder on germination, haustorium and radical length of broomrapes

Source of variation	*O.crenata*				*P.ramosa*			
	DF	**G**	**H**	**R**	**DF**	**G**	**H**	**R**
Sv	1	10.28**	3.03*	5.75*	1	21.19**	1.05ns	0.12ns
Level	5	1.06ns	0.66ns	1.27ns	5	3.85*	1.13ns	0.92ns
Sv*level	5	3.80*	1.04ns	2.45*	5	0.29ns	0.56ns	0.58ns
L S D		2.53	23.35	0.73		6.05	6.27	0.37

*=P<0.05, **=P<0.01, NS = not significantly, sv= safflower variety, G=germination, H= haustorium R= radical length

Appendix 2

Table (2): Effects of safflower stem powder on germination, haustorium and radical length of broomrape

Source of variation	*O.crenata*				*P.ramosa*			
	DF	**G**	**H**	**R**	**DF**	**G**	**H**	**R**
Sv	1	1.91ns	3.87*	0.62ns	1	3.05*	17..64**	0.66**
Level	5	2.69*	1.14ns	1.61ns	5	1.17ns	2.65*	0.45*
Sv*level	5	1.56ns	0.60ns	1.00ns	5	0.97ns	0.36ns	0.26ns
L S D		4.29	16.69	0.65		6.01	13.29	0.60

*=P<0.05, **=P<0.01, NS = not significantly, sv= safflower variety, G=germination, H= haustorium R= radical length

Appendix 3

Table (3): Effects of safflower leaves powder on germination, houstorum and radical length of broomrape

Source of variation	*O.crenata*				*P.ramosa*			
	DF	**G**	**H**	**R**	**DF**	**G**	**H**	**R**
Sv	1	14.16^{**}	0.40^{ns}	1.87^{ns}	1	142.58^{**}	5.49^{*}	0.63^{ns}
Level	5	1.78^{ns}	0.82^{ns}	1.74^{ns}	5	0.64^{ns}	0.73^{ns}	0.87^{ns}
Sv*level	5	$2..64^{*}$	1.46^{ns}	0.39^{ns}	5	3.39^{*}	2.97^{*}	0.72^{ns}
L S D		6.55	3.93	0.45		6.07	12.30	0.35

*=P<0.05, **=P<0.01, ^{NS} = not significantly, sv= safflower variety, G=germination, H= haustorium R= radical length