PLANT SCIENCE TODAY, 2021
Vol 8(2): 255–262
https://doi.org/10.14719/pst.2021.8.2.1007
ISSN 2348-1900 (online)

HORIZON
e-Publishing Group
HePG

RESEARCH COMMUNICATION

Efficacy of fungicides, plant extracts and biocontrol agents against Ascochyta blight (*Ascochyta rabiei*) of chickpea (*Cicer arietinum* L.) under field conditions

Salman Ahmad[1*], Muhammad Aslam Khan[2], Irfan Ahmad[3], Zafar Iqbal[1], Ejaz Ashraf[1], Muhammad Atiq[2], Yasir Ali[4] & Saima Naseer[5]

[1]College of Agriculture, University of Sargodha, Sargodha, Pakistan
[2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan
[3]Department of Forestry and Range Management, University of Agriculture, Faisalabad, Pakistan
[4]College of Agriculture, Bahadur Sub-Campus Layyah, Bahauddin Zakariya University, Multan, Pakistan
[5]Plant Pathology Research Institute, Ayub Agriculture Research Institute, Faisalabad, Pakistan
Email: salman.ahmad@uos.edu.pk

ARTICLE HISTORY
Received: 25 October 2020
Accepted: 03 January 2021
Published: 01 April 2021

KEYWORDS
Biocontrol agent
Ascochyta blight
Chickpea
Fungicides
Plant extracts

ABSTRACT

Two fungicides, Aliette and Thiovit$_{Jet}$ @ 0.15%, containing Aluminum tris (O-ethyl phosphonate) and sulphur compounds, respectively; two plant extracts, *Melia azedarach* and *Azadirachta indica* @ 8% and one biocontrol agent, *Trichoderma harzianum* @ 10^7 conidia ml^{-1} were investigated against ascochyta blight of chickpea under field conditions. Treatments were evaluated on three varieties susceptible to chickpea blight. Field trial revealed that Aliette and Thiovit$_{Jet}$ significantly decreased disease severity to 17 and 23% respectively, followed by *M. azedarach* and *A. indica* which decreased severity to 50 and 56% respectively, compared to control with 75% disease severity. *T. harzianum*, with a severity of 63%, was significantly less effective than fungicides and both plant extracts in controlling blight disease. The current research revealed that systemic and sulphur containing fungicides, both plant extracts and the biocontrol agent have the potential to control ascochyta blight of chickpea.

Introduction

Chickpea (*Cicer arietinum* L.) is a vital legume crop of the world, grown in more than fifty countries and is on third position in production after dry and field peas (1, 2). It is the source of high quality protein for humans and its crop residues are being used for animal feed. Chickpea contributes towards soil fertility in cereal-legume crop rotations (3). Chickpea are the most cultivated crop among legumes in Pakistan with annual production 359 thousand tons (4). Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Lab. (teleomorph: *Didymella rabiei*) (Kovachevski) v. Arx, is a serious constraint to chickpea production. The disease can cause complete crop loss under its epiphytotic occurrence (3). Annually, chickpea blight causes heavy yield losses in Pakistan (5) and caused serious economic losses during the 1980-84 epidemics (6).

Several fungicides have been reported effective in the world for the control of ascochyta blight but their repeated applications are uneconomical where chickpea yield is low (3, 7). Antracol, chlorothalonil, maneb, zineb, penconazole, propiconazole, thiabendazole, sulphur based fungicides and captan have been reported effective to avoid secondary spread of ascochyta blight (3). Recently, several plant extracts, viz., *Aloe vera*, *Magnolia grandiflora* and *Tagetes erectus* etc., have been tested and found effective against many plant diseases (8, 9). Plants have secondary metabolites with antifungal activity (10). Similarly, biocontrol agents *Chaetomium globosum*, *Trichoderma viride*, *Acremonium implicatum* have been reported *in vitro* for their effectiveness against ascochyta blight (11, 12).

To cite this article: Ahmad S, Khan M A, Ahmad I, Iqbal Z, Ashraf E, Atiq M, Ali Y, Naseer S. Efficacy of fungicides, plant extracts and biocontrol agents against Ascochyta blight (*Ascochyta rabiei*) of chickpea (*Cicer arietinum* L.) under field conditions. Plant Science Today. 2021;8(2):255–262. https://doi.org/10.14719/pst.2021.8.2.1007

Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, etc.
Full list at http://www.plantsciencetoday.online

Most indigenous chickpea germplasm of Pakistan is susceptible to chickpea blight (13). Under these circumstances, repeated applications of fungicides are made to protect the crop (14). Sometimes, the number of sprays may exceed to five time applications if the weather remains conducive to disease development for a longer period of time or inappropriate fungicides are applied (15). As a result, excessive use of chemicals is producing new pathotypes and polluting the environment. As residual effects of these fungicides are hazardous to health, there is need to select the most natural fungicides with eco-friendly alternatives including the plant extracts and biocontrol agents, which may reduce the use of chemicals (9, 16). By including the most suitable fungicides and natural sources, a sustainable management scheme can be developed to prevent future epidemics of chickpea blight and to avert problems associated with fungicide use. *In vitro* studies on the efficacy of plant extracts and biological control agents against blight have been reported (17, 18, 19). However, their effectiveness has not been checked under field conditions according to our best information. Hence, the present study was designed with an objective to evaluate the efficacy of fungicides, plant extracts and a biocontrol agent against chickpea blight *in vivo* conditions.

Materials and Methods

A preliminary experiment was conducted *in vitro* to find most effective fungicides, plant extracts and biocontrol agent along with their best doses.

Isolation, purification and identification of A. rabiei

Chickpea blight infected pods of cultivar Pb-1 were collected from Ayub Agricultural Research Institute (ARRI) located near to University of Agriculture, Faisalabad, Pakistan. The pods were placed in a refrigerator at 5-8 °C and used for isolation and purification of *A. rabiei* (20). *A. rabiei* was isolated on chickpea seed meal agar medium (CSMA) containing 20 g l^{-1} chickpea seed meal, 20 g l^{-1} agar and 20 g l^{-1} glucose by the standard procedure (20). Pods were placed in forceps grip and heated on sprit lamp flame in a way that outer surface of pods could be sterilized, while inner pod layer remains undamaged. Surface sterilized pods were then opened and infected seeds were brought out from the pods with sterilized forceps. Seven seeds were then placed in each petri-dish (100 mm × 15 mm) containing autoclaved CSMA medium and incubated at 20 ± 2 °C for 14 days (20). When colonies of *A. rabiei* formed around the plated infected material on CSMA medium, they were isolated, and purified by the single spore culture method (21).

In vitro evaluation of fungicides and plant extracts against A. rabiei

Five fungicides and five plant extracts (Table 1, 2) were tested at 0.05, 0.10, 0.15 and 3, 5 and 8 percent concentrations respectively, through poisoned food technique (23). To make the required percent concentrations of fungicides, amount of each fungicide (50, 100 and 150 mg) was weighed and dissolved in 100 ml distilled water. For the preparation of aqueous plant extracts, actively growing leaves/cloves were taken and surface sterilized with 1% sodium hypochlorite solution, then, thoroughly washed with distilled sterilized water. After that, the material was dried at 40 °C in an oven and then grinded in electric grinder. This ground material was then soaked in sufficient amount of sterilized water to get 20% W/V concentration of aqueous extract. After that, concentrated solution of plant extracts was filtered through muslin cloth and filter papers. Plant extracts were stored at 4 ^{0}C and used within four days to ensure the antifungal efficacy. The required concentrations of plant extracts were made in distilled sterilized water (19).

After that, petri-dishes were prepared by saturating fungicides/plant extracts in the CSMA medium in laminar flow chamber to ensure aseptic conditions (45). No fungicide/plant extract was applied in control plates. Disks of 7 mm *A. rabiei* culture with sterile cork borer were taken and punched in the center of each CSMA plate containing fungicides/plant extracts and in control plates. The plates were then kept in an incubator at 20 ± 2 °C until full growth of *A. rabiei* appeared in the control plate (having no fungicide/plant extract) (Fig. 1). The present studies on *in vitro* bioassay of fungicides/plant extracts were accomplished using completely randomized design (CRD) with three replications within each treatment. Percentage inhibition of mycelium growth was recorded by measuring colony diameter of treatments and control plates by using following formula (46):

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control} - \text{Growth in fungicidal treatment}}{\text{Growth in control}} \times 100$$

In vitro evaluation of biocontrol agents against A. rabiei

Strains PTF-0051 and E58 of biocontrol agents *Aspergillus flavus* and *T. harzianum* respectively were evaluated *in vitro* against *A. rabiei*. Strain PTF-0051 was purchased from Fungal Culture Bank of University of the Punjab, Lahore, Pakistan, while strain E58 was kindly provided by Biochemistry Department, University of Agriculture, Faisalabad, Pakistan. Spore concentrations 10^5, 10^6 and 10^7 conidia ml^{-1}, of these strains of biocontrol agents (*A. flavus* and *T. harziaum*) were made in distilled sterilized water with the help of haemocytometer, and effectiveness was checked by dual culture assay (17). The biocontrol fungal strains were grown on potato dextrose agar medium in petri-dishes and incubated at 25 °C. For dual culture, 7 mm disk of fungal culture of *A. rabiei* was taken and put on one side of the petri-plate, then with sterilized syringe the required concentration of antagonists was taken and placed on the other side of the plate. The plates were then placed in incubator at 20 ± 2 °C until the full growth appeared in the control. In this study, CRD design was used and each treatment was replicated thrice. The percentage inhibition of *A. rabiei* was determined by following formula (44).

$$\text{Percentage Inhibition} = \frac{\text{C-T} \times 100}{\text{C}}$$

C = Radial growth in control
T = Radial growth in treatment

The data were subjected to analysis of variance (ANOVA) for the determination of main and interactive effects of treatments. For the comparison of means, least significant difference (LSD) test at 0.05 was used (28).

Streptomycin (50 mg) was mixed with the autoclaved seeds to avoid bacterial contamination. These cultures were then incubated at 20 ± 2 °C for 10 days for further growth of the pycnidial culture of *A. rabiei* (22).

Table 1. Details of fungicides used in research trials

Sl. No.	Trade Name	Common Name	Chemical Group	Active Ingredient	Formulation	Manufacturer
1.	Aliette WG	Aliette	Organophosphate	Aluminum tris (O-ethyl phosphonate)	Water dispersible granule	Bayer Crop Science, Karachi, Pakistan
2.	Thiovit Jet	Lime sulphur	Inorganic sulphur compounds	Sulphur 800 g/kg	Water dispersible granule	Syngenta Pakistan Limited, Karachi, Pakistan
3.	Cabrio Top 60WDG	$Cabrio_{Top}$	Pyraclostrobin & Metiram	Pyraclostrobin 5% + Metiram 55%	Water dispersible granule	FMC United Private Limited, Lahore, Pakistan
4.	Nativo WG (75 WG)	Nativo	Tuboconazole	Tebuconazole 50%+ Trifloxystrobin 25% w/w	Water dispersible granule	Bayer Crop Science, Karachi, Pakistan
5.	Antracol	Propineb	Ethylenebisdithiocarbamates	Propineb 700 g/kg	Wettable powder	Bayer Crop Science, Karachi, Pakistan

Table 2. Plant materials used against *A. rabiei*

Sl. No.	Common Name	Botanical Name	Family	Parts used
1	Neem	*Azadirachta indica* A. Juss.	Meliaceae	Leaves
2	Garlic	*Allium sativum* L.	Alliaceae	Cloves
3	Datura	*Datura stramonium* L.	Solanaceae	Leaves
4	Bakain	*Melia azedarach*	Meliaceae	Leaves
5	Ak, Akund	*Calotropis procera* Wild. Drayandex. W. Ait.	Asclepiadiaceae	Leaves

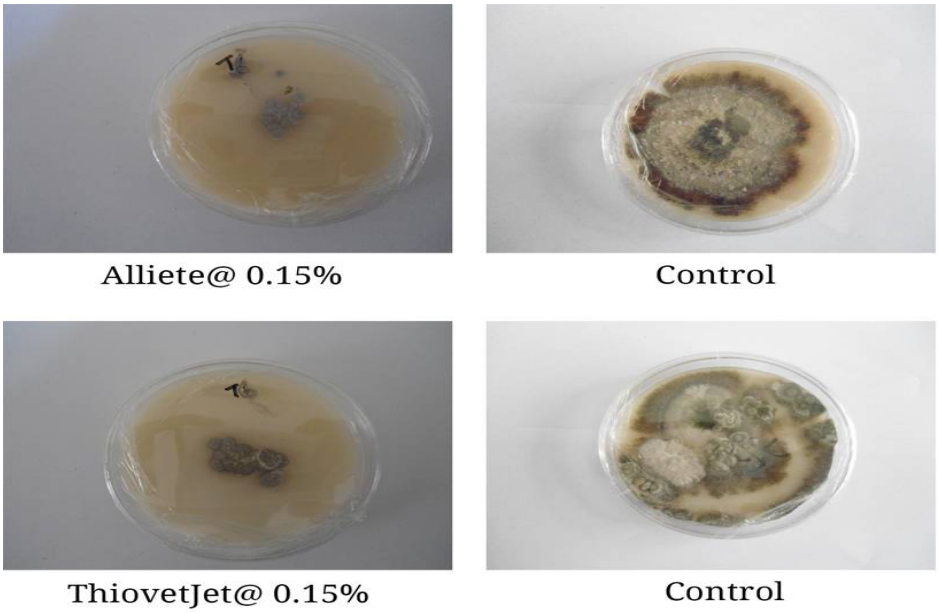

Fig. 1. Inhibition of colony growth of *A. rabiei* by two fungicides using poisoned food technique.

Preparation of mass culture of A. rabiei

The materials used for the preparation of mass culture of *A. rabiei* were 30 x 24 cm size polypropylene bags, 2.5 cm plastic of the same diameter, cotton plugs and chickpea seeds and was prepared by the standard method (22). For this purpose, chickpea seeds were soaked in tap water for overnight, after that spread on paper towels to remove free moisture and were surface dried. Surface dried seeds @ 500 gm bag^{-1} were placed in polypropylene bags and open end was fixed with cotton plugs. These bags were autoclaved at 121 °C and 138 KPa for 30 min, again sterilized after 24 h, and inoculated with three or four 6 mm agar plugs from a 14 day old culture (having maximum sporulation 1 × 10^6 conidia/ml) of *A. rabiei* (22).

Evaluation of fungicides, plant extracts and biocontrol agent against A. rabiei under field conditions

The most effective fungicides, plant extracts and biocontrol agent and their most effective doses, were evaluated under filed conditions. The treatments used in the present study under field conditions were: T_0 = Control (water), T_1 = Aliette (0.15%), T_2 = $Thiovit_{Jet,}$ (0.15%), T_3 = *M. azedarach* (8%), T_4 = *A. indica* (8%), T_5 = *T. harzianum* (10^7 conidia/ml^{-1}).

The study was conducted at the research area of University of Agriculture, Faisalabad, situated in semi-arid climate of province, Punjab, Pakistan (73°74 East, 30°31.5 North and 184 m above sea level). The research area is used for research trials on which different crops are grown. Previously sown crop on

this area was cotton. Prior to conducting this study, basic soil characteristics were determined (sand 45%; silt 31%; clay 23%; texture class was loam; bulk density 1.45 Mg ha^{-1}; saturated soil water content 45%; soil effective porosity 40%; and soil organic carbon 1.90 g Kg^{-1}). Recommended agronomic practices were performed during the course of experiment (26). Three chickpea varieties, CM-2000, CM-98 and Pb-1, highly susceptible to chickpea blight, were sown in Randomized Complete Block Design (RCBD) with three replications during both trials (Fig. 2). Each line was sown in row of 3 meter length with row to row and plant to plant distance of 30 cm and 15 cm, respectively. In each block, there were 16 rows of one variety, 15 for treatments and one as a control. All the blocks were sprayed with inoculum of *A. rabiei* (5×10^5 conidia/ml^{-1}) until the epidemic conditions prevailed in the blocks (Fig. 3) (47). After that, the solutions of fungicides Aliette and $Thiovit_{Jet}$ were prepared by dissolving 1.5 gm of each fungicide in one liter of water. Similarly, 80 ml filtrate solution of both plant extracts of *M. azedarach* and *A. indica* was taken from the refrigerator which were stored at 4 °C and dissolved in one liter of sterilized water to make required formulations. When disease started to appear on 10 weeks old plants, the treatments were applied with a knapsack sprayer. Three treatment sprays were applied at an interval of seven days on each variety in each block in such a way that first five rows were sprayed with all treatments first, second five rows after seven days and last five rows after fourteen days. Only distilled water was sprayed on the control. The disease severity index (DSI) was calculated for 10 plants per replicate at maturity by the standard formula (48).

$$\text{DSI (\%)} = \frac{\text{Total of all ratings}}{\text{No. of plants examined}} \times \frac{100}{\text{Max. disease rating}}$$

Disease ratings of chickpea blight disease severity were taken using a 1-10 modified rating scale (26) (Table 9). Data was subjected to ANOVA to determine the main and interactive effects of treatments while LSD test was used to assess the significance of differences between treatment means (28). During current research statistical software Minitab ver.17 was used (49).

Results and Discussion

ANOVA of *in vitro* evaluation of fungicidal treatments indicated that the individual effects of treatments and concentrations were significant. Two-way interactive effects of treatments and concentrations were also significant (Table 3). Fungicides at concentrations, i.e., 0.05%, 0.01% and 0.15%, significantly decreased colony growth of *A. rabiei* compared to control. Out of five fungicides, two fungicides Aliette and $Thiovit_{Jet}$ were significantly superior to other fungicides. Aliette inhibited colony growth to 87% at 0.15%, to 84% at 0.10% and to 81% at 0.05% respectively. Similarly, $Thiovit_{Jet}$ exhibited 83% inhibition at 0.15% than 0.10 (78%) and 0.05 (74%) respectively. Fungicide Antracol was found significantly less effective against *A. rabiei* compared to other treatments at different concentrations (Table 4).

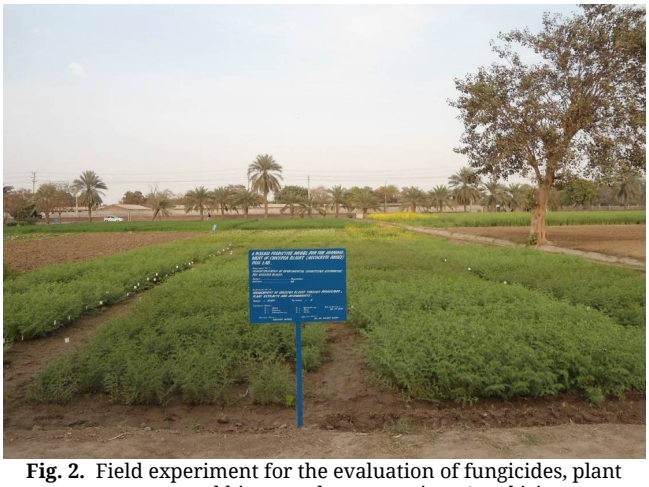

Fig. 2. Field experiment for the evaluation of fungicides, plant extracts and biocontrol agent against *A. rabiei*.

ANOVA of *in vitro* evaluation of plant extracts showed that the individual effects of treatments and concentrations were significant. Two-way interaction between treatments and concentrations was also significant (Table 5). Plant extracts at different concentrations significantly affected colony growth. Plant extracts of *M. azedarach* and *A. indica* performed the best compared to other plant extracts. *M. azedarach* and *A. indica* at 2%, 3% and 8% concentrations inhibited colony growth of *A. rabiei* to 37%, 42% and 47% and 20%, 25% and 36%, respectively (Table 6). Plant extracts of *A. sativum* and *D. stramonium* remained second good. Significantly less percentage inhibition of colony was

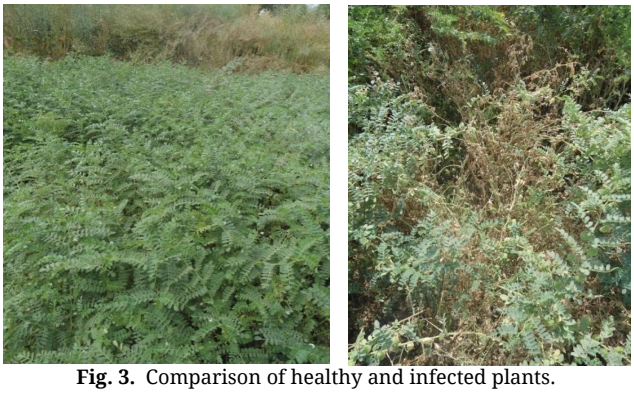

Fig. 3. Comparison of healthy and infected plants.

recorded in terms of *C. procera*. All plant extracts were significantly more active at their higher concentrations (Table 6).

ANOVA of *in vitro* evaluation of biocontrol agents showed that individual effects of treatments and concentrations of biocontrol agents were significant. Interactive effects of treatments and concentrations were also significant (Table 7). Both biocontrols, *T. harzianum* and *A. flavus*, showed significantly more inhibition of colony growth at higher spore concentrations than low (Table 8). *Ascochyta flavus* at spore concentration of 10^7 conidia ml^{-1} and *T. harzianum* at 10^6 conidia ml^{-1}showed statistically same ($P \leq 0.05$) percent inhibition. *T.*

Table 9. Chickpea blight disease rating scale

Infection (%)	1-10 Point scale	Symptoms	Reaction
1-10	1-<2	No infection or small lesions	Highly resistant
11-20	2-<3	Some stem lesions -minor stem breakage in upper foliage	Resistant
21-30	3-<4	One or two branches broken. Several girdling stem lesions low down on some branches	Resistant
31-40	4-<5	Large basal stem lesions or several branches broken near to main stem	Moderately resistant
41-50	5-<6	Half foliage dead	Moderately resistant
51-60	6-<7	Half foliage dead, but young shoots still actively growing from base	Moderately susceptible
61-70	7-<8	Most foliage dead. Some healthy stem tissue with lateral buds	Susceptible
71-80	8-<9	Most foliage dead, no healthy lateral buds in leaf axils	Susceptible
81-90	9-<10	Most foliage dead, decreasing areas of living stem tissue	Highly susceptible
91-100	10	Plants completely dead	Highly susceptible

harzianum at spore concentrations 10^5, 10^6 and 10^7 conidia ml^{-1} inhibited colony growth to 17%, 28% and 41%, respectively. While, *A. flavus* at spore concentrations of 10^5, 10^6 and 10^7 conidia ml^{-1} showed 9%, 20% and 30% decrease in colony growth respectively.

ANOVA of field evaluation of treatments under field conditions showed that two-way interactive effects of years and treatments, sprays and varieties, sprays and treatments and varieties and treatments were also significant. However, two-way interactive effects of years and sprays and years and varieties were not significant. Three-way interactive effects of years, varieties and treatments and sprays, varieties and treatments were significant but three way interactive effects of years, sprays and varieties and years, sprays and treatments were not significant.

Table 3. ANOVA for the effect of different concentrations of fungicides on colony growth inhibition of *A. rabiei*

S.O.V	DF	SS	MS	F	Pr> F
Replication	2	11.0	5.5		
Treatments	4	4291.2	1072.8	626.21	0.0001*
Concentrations	3	52843.0	17614.3	10281.6	0.0001*
Treatments × Concentrations	12	1795.5	149.6	87.34	0.0001*
Error	38	65.1	1.7		
Total	59	59005.9			

*Significant at 0.05 difference; CV = 5.27; Ns = non-significant; DF = Degree of freedom; SS = sum of squares; MS = Mean square

Table 4. Evaluation of different concentrations of five fungicides against colony growth of *A. rabiei*

Treatments	Concentrations		
	0.05%	0.10%	0.15%
T1=Cabrio$_{Top}$	53* i	62 h	75 f
T2=Thiovit$_{Jet}$	74 e	78 d	83 b
T3= Aliette	81 c	84 b	87 a
T4=Antracol	43 k	48 j	63 g
T5=Nativo	63 g	65 g	70 f
Untreated	0.0 l	0.0 l	0.0 l

LSD value = 3.32; Mean values sharing similar letters in a column do not differ significantly as determined by the LSD test at 5% level of probability

Four-way interactive effects of years, sprays, varieties and treatments were also not significant (Table 10).

Disease severity was significantly reduced by fungicides i.e. Aliette (17%) and Thiovit$_{Jet}$ (23%) followed by two plant extracts *M. azedarach* and *A. indica* reduced disease severity by 50% and 56%, respectively compared to control (75%). Biocontrol agent i.e. *T. harzianum* was significantly less effective (63%) in controlling blight disease severity compared to fungicides and both plant extracts (Table 11).

Three way interactions among treatments, years and sprays showed that treatments inhibited disease severity significantly as compared to control in both years. There was statistically significant difference in disease severity control by different treatments (Table 12). Significant disease control was observed in second year trial by all treatments than first year trial. Fungicides proved best during both years followed by both plant extracts and a biocontrol agent (Table 12). Three-way interaction revealed that disease severity decreased with increasing number of sprays (Table 12). Effect of Aliette, Thiovit$_{Jet}$, *M. azedarach* and *A. indica* on disease severity control was not statistically similar at different sprays. However, effect of *T. harzianum* at second and third spray was statistically significant at par. Third spray of all treatments controlled disease severity significantly as compared to first and second spray (Table 12).

Table 5. ANOVA for the effect of different concentrations of plant extracts on colony growth inhibition of *A. rabiei*

	DF	SS	MS	F	Pr> F
Replication	2	7.7	3.87		
Treatments	4	5407.7	1351.92	522.89	0.0001*
Concentrations	3	5566.3	1855.44	717.65	0.0001*
Treatments × Concentrations	12	1922.6	160.22	61.97	0.0001*
Error	38	98.2	2.59		
Total	59	13002.6			

*Significant at 0.05 difference; CV = 10.20; Ns = non-significant; DF = Degree of freedom; SS = sum of squares; MS = Mean square

Table 6. Evaluation of different concentrations of five plant extracts against colony growth of *A. rabiei*

Treatments	Concentrations		
	3%	5%	8%
T1= *M. azedarach*	37* c	42 b	47 a
T2= *A. indica*	20 e	25 d	36 c
T3= *A. sativum*	13 g	19 f	24 e
T4= *D. stramonium*	10 h	14 g	15 f
T5= *C. procera*	5. i	7 ij	8 hi
Untreated	0.0 k	0.0 k	0.0 k

LSD value = 2.97; Mean values sharing similar letters in a column do not differ significantly as determined by the LSD test at 5% level of probability

Three way interactions among treatments, varieties and sprays indicated that control of chickpea blight disease severity at spray first, second and third of fungicides on varieties CM-2000 and Pb-1 was statistically at par except CM-98 (Table 13). While, effects of both plant extracts and a biocontrol agent on chickpea blight disease control were statistically not at par on three varieties. Fungicides proved best in reducing disease on all three varieties followed by plant extracts compared to control. *T. harzianum* was found significantly less effective on three varieties in controlling disease severity (Table 13).

Table 7. ANOVA for the effect of different concentrations of biocontrol agents on colony growth inhibition of *A. rabiei*

S.O.V	DF	SS	MS	F	Pr> F
Replication	2	43.28	21.64		
Treatments	1	296.18	296.18	85.58	0.0001*
Concentrations	3	4026.87	1342.29	387.86	0.0001*
Treatments × Concentrations	3	108.93	36.31	10.49	0.0007*
Error	14	48.45	3.46		
Total	23	4523.71			

*Significant at 0.05 difference; CV= 10.21; Ns =non-significant; DF = Degree of freedom; SS = sum of squares; MS = Mean square

Table 8. Evaluation of different concentrations of two biocontrol agents against colony growth of *A. rabiei*

Treatments	Dose	% Inhibition
T1= *A. flavus*	1x10^5 conidia mL^{-1}	9* d
T2= *A. flavus*	1x 10^6conidia mL^{-1}	20 c
T3= *A. flavus*	1x 10^7conidia mL^{-1}	30 b
T4= Untreated	00	0.0 e
T1= *T. harzianum*	1x 10^5 conidia mL^{-1}	17 c
T2= *T. harzianum*	1x 10^6conidia mL^{-1}	28 b
T3= *T. harzianum*	1x 10^7conidia mL^{-1}	41 a
T4= Untreated	00	0.0 e

LSD value = 4.43; Mean values sharing similar letters in a column do not differ significantly as determined by the LSD test at 5% level of probability

Significant control of chickpea blight by Aliette fungicide *in vivo* conditions is due to its systemic ability which allowed this fungicide to kill the fungus in established infection. This is in line with the findings of (20, 26, 29-31). During this study, maximum control of chickpea blight was obtained using three foliar applications of Aliette. It was reported that with fewer applications of curative systemic fungicides, ascochyta blight can be controlled effectively on chickpea crop (50). Successful control of ascochyta blight through this systemic fungicide might also have resulted because of its good translocation into tissues of the host. Previous researches have shown that only those systemic fungicides perform well which show movement into newly developed tissues (34). Thus, the present study recommends that continuous efforts should be made to look for those systemic fungicides which show more translocation in the system of the plant. Similarly, Thiovit$_J$et with three applications also effectively controlled the disease; and is attributed to the antifungal activity of its active ingredient sulphur (35). Sulphur containing fungicides are affective against chickpea blight (3),

Table 10. ANOVA of fungicides, plant extracts and biocontrol agent to control chickpea blight disease under field conditions

Source of variation	DF	SS	MS	F	*P*
Replication	2	103	51.4		
Years	1	1985	1985.2	452.65	0.0001*
Sprays	2	5280	2639.8	601.92	0.0001*
Varieties	2	12418	6209.0	1415.75	0.0001*
Treatments	5	142248	28449.6	6486.93	0.0001*
Years x sprays	2	14	7.05	1.60	0.2111ns
Years x Varieties	2	1	0.5	0.10	0.9011ns
Years x Treatments	5	517	103.4	23.58	0.0001*
Sprays x Varieties	4	665	166.2	37.90	0.0026*
Sprays x Treatments	10	1379	137.9	31.44	0.0001*
Varieties x Treatments	10	4451	445.1	101.49	0.0001*
Years x Sprays x Varieties	4	11	2.7	0.62	0.6492ns
Years x Sprays x Treatments	10	73	7.3	1.67	0.0886ns
Years x Varieties x Treatments	10	114	11.4	2.60	0.0054*
Sprays x Varieties x Treatments	20	270	13.5	3.08	0.0001*
Years x Sprays x Varieties x Treatments	20	46	2.3	0.52	0.9555ns
Error	214	939	4.4		

*Significant at 0.05 difference; CV= 14.41; Ns =non-significant; DF = Degree of freedom; SS = sum of squares; MS = Mean square

Table 11. Evaluation of fungicides, plant extracts and biocontrol agent to control chickpea blight disease under field conditions

Sl. No.	Treatments	Mean values of chickpea blight percent disease severity
T_1	Aliette @ 0.15%	17±0.56^{f*}
T_2	Thiovit$_{Jet}$ @ 0.15%	23±1.25^e
T_4	*M. azedarach* @ 8%	50±3.89^d
T_3	*A. indica* A. Juss.@ 8%	56±4.47^c
T_5	*T. harzianum* @ 1x 10^7	63±1.11^b
T_0	Control	75±0.67^a
L.S.D.	1	

*Means with similar letters are not significantly different at *P* = 0.05

and avoid resistance in fungal pathogens due to their multiple site mode of action (35). Thus, present management programme suggests use of systemic fungicides with rotation of sulphur based fungicides to control chickpea blight.

Disease control by plant extracts may be ascribed to their ability of inducing Systemic Acquired Resistance (SAR) (36). Foliar application of *A. indica* produces SAR effectively in chickpea cultivars against ascochyta blight (37). Plant extracts may also control the disease by different antifungal compounds which they contain. It was found that *M. azedarach* contained benzoic acid, ursolic acid, maesol, 3,5 dimethoxybenzoic acid, ß-sitosterol and ß-amyrin which were highly toxic to chickpea blight fungus (9). Similarly, from *A. indica*, obacunone, nomilin, limonoids and limonin have been isolated and proved to be effective against different insects and fungi (38). Botanical extracts also contain

Table 12. Interaction effects of treatments, years and sprays of fungicides, plant extracts and biocontrol agent to control chickpea blight disease under field conditions

Treatments	1st Year Trial			2nd Year Trial		
	Spray1	Spray2	Spray3	Spray1	Spray2	Spray3
Aliette @ 0.15%	29 ±0.23^{k*}	21±0.96^{l}	14±0.43^{m}	19±1.01^{l}	13±1.10^{m}	9±0.70^{n}
Thiovit$_{Jet}$ @ 0.15%	34±1.58^{j}	25±2.01^{k}	19±1.27^{l}	26±1.61^{k}	18±1.93^{l}	13±1.25^{m}
M. azedarach @ 8%	60±4.85^{ef}	52±3.68^{g}	46±3.99^{h}	53±4.09^{g}	45±3.55^{h}	41±3.01^{i}
A. indica A. Juss.@ 8%	66±2.88^{bc}	59±1.74^{f}	53±1.05^{g}	60±3.01^{ef}	53±2.23^{g}	48±2.69^{h}
T. harzianum @ 1x 10^7	69±0.90^{b}	63±0.62^{cde}	63±0.74^{cde}	65±1.06^{cd}	61±0.53^{def}	60±0.54^{ef}
Control	76±0.20^{a}	74±0.40^{a}	77±0.25^{a}	77±0.55^{a}	74±0.67^{a}	75±0.89^{a}
L.S.D	11	11	11	11	11	11
L.S.D		6			6	

*Means with similar letters in a row are not significantly different at $P = 0.05$.

Table 13. Interaction effects of treatments, varieties and sprays of fungicides, plant extracts and biocontrol agent to control chickpea blight disease under field conditions

Treatments	CM-2000			CM-98			Pb-1		
	Spray1	Spray2	Spray3	Spray1	Spray2	Spray3	Spray1	Spray2	Spray3
Aliette @ 0.15%	22±0.29^{tuv*}	13±2.96^{xy}	7±3.89^{z}	30±0.96^{r}	20±3.15^{uvw}	14±3.37^{xy}	20±2.51^{vw}	17±2.41^{wxy}	12±4.04^{yz}
Thiovit$_{Jet}$ @ 0.15%	28±2.68^{rst}	17±3.07^{vwx}	13±4.02^{xy}	37±1.90^{q}	26±1.82^{rst}	19±1.68^{vw}	25±1.72^{stu}	21±1.86^{uvw}	17±1.34^{wxy}
M. azedarach @ 8%	56±2.52^{jkl}	46±2.46^{no}	42±3.60^{op}	67±2.25^{fgh}	57±4.13^{jkl}	49±3.19^{mn}	47±2.06^{no}	44±3.19^{o}	39±2.18^{pq}
A. indica A. Juss. @ 8%	67±3.94^{fgh}	55±4.88^{kl}	52±5.06^{lm}	73±2.96^{de}	65±2.78^{hi}	56±2.14^{jkl}	49±2.30^{mn}	47±2.37^{no}	43±2.90^{op}
T. harzianum @ 1x 10^7	71±0.92^{efg}	66±0.75^{h}	66±1.24^{gh}	77±2.36^{cd}	71±2.58^{ef}	69±1.27^{efgh}	53±0.96^{lm}	50±2.05^{mn}	47±2.15^{no}
Control	81±2.66^{abc}	78±3.93^{bc}	79±4.69^{bc}	86±4.20^{a}	85±3.17^{a}	83±2.86^{ab}	61±3.05^{ij}	58±3.70^{jk}	66±3.22^{h}
L.S.D	5	5	3	5	6	4	5	4	5
L.S.D		4			5			3	

* Means with similar letters in a row are not significantly different at $P = 0.05$.

secondary metabolites which are antifungal and restrict the mycelial growth of the fungi (39).

Disease control by biocontrol agent *T. harzianum* may be due to the enzymes produced by this biocontrol agent. Chitinase β-1, 3-glucanase, protease and xylase have been reported to be produced by biocontrol agents (40, 41). While, under field conditions, biological organisms confer resistance in plants by stimulating their defense mechanisms and thus, play role in disease control (42).

Conclusion

The current research has shown that systemic and sulphur containing fungicides can be used for curative applications to control chickpea blight. Further, mild severities of chickpea blight can be controlled using plant extracts and biocontrol.

Authors' contributions

All the authors jointly conceptualized the experimental strategy and financial support. They supported in field data collection, laboratory works, compilation, analyses, manuscript writing, submission, correction etc.

Conflict of interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

References

1. FAOSTAT. Food and Agricultural Organization (FAO), Bulletin of Statistics. Crop. Production. 2017. http://www.faostat.fao.org
2. Tadesse M, Turoop L, Ojiewo CO. Survey of Chickpea (*Cicer arietinum* L.) Ascochyta Blight (*Ascochyta rabiei* Pass.) disease status in production regions of Ethiopia. Plant Science. 2017;5(1):22-30. http://oar.icrisat.org/id/eprint/9971
3. Pande S, Siddique KH, Kishore GK, Bayaa B, Gaur PM, Gowda CL, Bretag TW, Crouch JH. Ascochyta blight of chickpea (*Cicer arietinum* L.): a review of biology, pathogenicity and disease management. Australian Journal of Agricultural Research. 2005;56(4):317-32. https://doi.org/10.1071/AR04143
4. GOP. Agricultural Statistics of Pakistan. Ministry of Food, Agriculture and Cooperatives, Govt. of Pakistan. Food and Agriculture Division, (Planning Unit), Islamabad. 2017.
5. Jamil FF, Sarwar MU, Sarwar NI, Khan JA, Zahid MH, Yousaf S, Arshad HM, Haq I. Genotyping with RAPD markers resolves pathotype diversity in the Ascochyta blight and Fusarium wilt pathogens of chickpea in Pakistan. Pakistan Journal of Botany. 2010;42(2):1369-78.
6. Malik BA, Bashir M. Strategies for controlling gram blight. Progressive farming. 1984; 4:21-23.
7. Banniza S, Armstrong-Cho CL, Gan Y, Chongo G. Evaluation of fungicide efficacy and application frequency for the control of ascochyta blight in chickpea. Canadian Journal of Plant Pathology. 2011;33(2):135-49. https://doi.org/10.1080/07060661.2011.561875
8. Hernandez-Terrones MG, Morais SA, Ferreira S, Santos DQ, Nascimento EA, Chang R. Phytochemistry and allelophatic study of *Pterodon emarginatus* stem extract. Planta daninha. 2007;25(4):755-62. http://dx.doi.org/10.1590/S0100-83582007000400012
9. Khajista J, Javaid A, Ahmad E, Athar M. Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei*, the cause of chickpea blight. Natural product research. 2011;25(3):264-76. https://doi.org/10.1080/14786411003754298
10. Lubian C, Martinha DD, Portz R, Gonçalves MP, Holz S, Marcelino WL, Nogueira AC, Thomé RM, Missio VC, Cordeiro J, Feroldi LT. Anthelmintic activity of plant aqueous extracts against *Panagrellus redivivus* in vitro. Arquivos do Instituto Biológico. 2019;86. https://doi.org/10.1590/1808-1657000672018
11. Bisen K, Singh V, Keswani C, Ray S, Sarma BK, Singh HB. Use of Biocontrol Agents for the Management of Seed-Borne Diseases. In Seed-Borne Diseases of Agricultural Crops: Detection, Diagnosis & Management. Springer, Singapore. 2020;651-63. https://doi.org/10.1007/978-981-32-9046-4_22

12. Agarwal T, Malhotra A, Trivedi PC, Biyani M. Biocontrol potential of *Gliocladium virens* against fungal pathogens isolated from chickpea, lentil and black gram seeds. Journal of Agricultural Technology. 2011;7(6):1833-39. http://www.ijat-aatsea.com ISSN 1686-9141
13. Abdul R, Muhammad UY, Muhammad EH, Iftikhar HW, Ejaz P, Faqir MA, Masood A. 2014. Screening of chickpea (*Cicer arietinum* L.) varieties against ascochyta blight and its management through bio-pesticides. Archives of Phytopathology and Plant Protection. 2014;12:938-43. https://doi.org/10.1080/03235408.2013.826540
14. Rani U, Singh S, Basandrai AK, Rathee VK, Tripathi K, Singh N, Dixit GP, Rana JC, Pandey S, Kumar A, Singh K. Identification of novel resistant sources for ascochyta blight (*Ascochyta rabiei*) in chickpea. Plos one, 2020;19:15(10):e0240589. https://doi.org/10.1371/journal.pone.0240589
15. Benzohra IE, Bendahmane BS, Benkada MY, Megateli M, Belaidi H. Use of three synthetic fungicides to reduce the incidence of ascochyta blight (*Ascochyta rabiei*) in chickpea (*Cicer arietinum* L.): A susceptible cultivars case. Indian Journal of Agricultural Research. 2020;54(4):459-64.
16. Leelavathi MS, Vani L, Reena P. Antimicrobial activity of *Trichoderma harzianum* against bacteria and fungi. International Journal of Current Microbiology and Applied Sciences, 2014;3(1):96-103.
17. Kucuk Ç, KivanÇ M, Kinaci E, Kinaci G. Efficacy of *Trichoderma harzianum* (Rifaii) on inhibition of ascochyta blight disease of chickpea. Annals of Microbiology. 2007;57(4):665-68. https://doi.org/10.1007/BF03175370
18. Shafique S, Shafique S. Antifungal activity of n-hexane extracts of *Datura metal* against *Ascochyta rabiei*. Mycopath. 2008;6:31-35.
19. Shafique S, Shafique S, Bajwa R, Akhtar N, Hanif S. Fungitoxic activity of aqueous and organic solvent extracts of *Tagetes erectus* on phytopathogenic fungus- *Ascochyta rabiei*. Pakistan Journal of Botany. 2011;43(1):59-64.
20. Ilyas MB, Iqbal A. Evaluation of fungicides for the eradication of seed borne *Ascochyta rabiei* fungus. Pakistan Journal of Agricultural Sciences. 1986;23(3-4):145-49.
21. Choi YW, Hyde KD, Ho WH. Single spore isolation of fungi. Fungal diversity, 1999;3:29-38.
22. Ilyas MB, Khan IU. A low cost easy technique for the culturing of *Ascochyta rabiei* fungus. Pakistan Journal of Agricultural Sciences. 1986:23:60.
23. Nene Y, Thapilyal L. Poisoned food technique of fungicides in plant disease control. 3rd edition. Oxford. New Delhi. 2002.
24. Mortan DT, Straube NH. Antagonistic and stimulatory effects of microorganisms *Sclerotium rolfsii*. Phytopathology. 1955;45:419-20.
25. Iqtidar AK, Amanullah J. Cropping Technology. National Book Foundation. Islamabad. 2002.
26. Ahmad S, Khan MA, Ahmad I, Ashraf E, Aatif HM, Ali A, Safdar ME, Anjum MZ, Raza W. Predictive Modeling for Chickpea Blight (*Ascochyta rabiei*) Occurrence in the Semi-Arid Zone Using Meteorological Data from Faisalabad, Pakistan. Philippine Agricultural Scientist. 2019;102(4):330-39.
27. Sambasivam PT, Mehmood Y, Bar I, Davidson J, Moore K, Hobson K, Ford R. Evidence of recent increased pathogenicity within the Australian *Ascochyta rabiei* population. BioRxiv. 2020; preprint doi: https://doi.org/10.1101/2020.06.28.175653
28. Steel RGO, Torrie JH, Dickey D. Principles and Procedures of Statistics: A Biometrical Approach. Mc Graw-Hill. New York, 1997.
29. Kukreja S, Salaria N, Thakur K, Goutam U. Fungal Disease Management in Chickpea: Current Status and Future Prospects. In Fungi and their Role in Sustainable Development: Current Perspectives, 2018;293-309. Springer, Singapore. https://doi.org/10.1007/978-981-13-0393-7_17
30. Chongo G, Banniza S, Gan Y, Wolf T, Warkentin T. Fungicides application timing, sequencing and tank mixing for controlling blight in chickpea. In Soils and Crops Workshop. 2003; p11-12. Saskatoon, Canada.
31. Chongo G, Buchwaldt L, Gossen BD, Lafond GP, May WE, Johnson EN, Hogg T. Foliar fungicides to manage ascochyta blight (*Ascochyta rabiei*) of chickpea in Canada. Canadian Journal of Plant Pathology. 2003;25(2):135-42. https://doi.org/10.1080/07060660309507061
32. Koleva M, Stanoeva Y, Kiryakov I, Ivanova A. Sources of resistance in chickpea (*Cicer arietinum* L.) to ascochyta blight (*Ascochyta rabiei*). Agricultural Science and Technology. 2018; 10(3): 195-98. https://doi.org/10.15547/ast.2018.03.037
33. Davidson JA, Kimber RBE. Integrated disease management of ascochyta blight in pulse crops. In: Tivoli B, Baranger A, Muehlbauer F. J, Cooke B.M. (eds) Ascochyta blights of grain legumes. Springer, Dordrecht. 2007; https://doi.org/10.1007/978-1-4020-6065-610
34. Shahid M, Khan MS, Zaidi A. Fungicide Toxicity to Legumes and Its Microbial Remediation: A Current Perspective. Pesticides in Crop Production: Physiological and Biochemical Action. 2020;6:15-33. https://doi.org/10.1002/9781119432241.ch2
35. Künstler A, Gullner G, Ádám AL, Kolozsváriné Nagy J, Király L. The Versatile Roles of Sulfur-Containing Biomolecules in Plant Defense—A Road to Disease Resistance. Plants, 2020;9(12):1705. https://doi.org/10.3390/plants9121705
36. Guleria S, Kumar A. *Azadirachta indica* leaf extract induces resistance in sesame against Alternaria leaf spot disease. Journal of Cell and Molecular Biology. 2006;5(2):81-86.
37. Sarwar N, Zahid MH, Shafaq A, Jamil FF. Induced systemic resistance in chickpea against ascochyta blight by safe chemicals. Pakistan Journal of Botany. 2011;43:1381-87.
38. Ilyas A, Khan HA, Qadir A. Effect of Essential Oils of some Indigenous Plants on Settling and Oviposition Responses of Peach Fruit Fly, *Bactrocera zonata* (Diptera: Tephritidae). Pakistan Journal of Zoology. 2017;49(5:1547-53.
39. Sisti MA, De Santi MA, Fraternale DA, Ninfali PA, Scoccianti VA, Brandi GI. Antifungal activity of *Rubus ulmifolius* Schott standardized *in vitro* culture. LWT-Food Science and Technology. 2008;41(5):946-50. https://doi.org/10.1016/j.lwt.2007.05.012
40. Sood M, Kapoor D, Kumar V, Sheteiwy MS, Ramakrishnan M, Landi M, Araniti F, Sharma A. *Trichoderma*: the "secrets" of a multitalented biocontrol agent. Plants. 2020;9(6):p.762. https://doi.org/10.3390/plants9060762
41. Javeria S, Kumar A, Kharkwal AC, Varma A, Srinivasa N, Sharma P. Evaluation of rhizospheric *Trichoderma* species strains for producing cell wall-degrading and defense related enzymes in response to *Fusarium oxysporum* f. sp. *lentis*. Indian Phytopathology. 2020:73(3):461-7. https://doi.org/10.1104/pp.108.130369
42. Dukare A, Paul S. Biological control of Fusarium wilt and growth promotion in pigeon pea (*Cajanus cajan*) by antagonistic rhizobacteria, displaying multiple modes of pathogen inhibition. Rhizosphere. 2020:100278. https://doi.org/10.1016/j.rhisph.2020.100278
43. Ferreira JHS, Matthee FN, Thomas AC. Biological control of Eutypalata on grapevine by an antagonistic strain of *Bacillus subtilis*. Phytopathology. 1991:81:283-87.
44. Küçük Ç, Kivanç M. Isolation of *Trichoderma* spp. and their antifungal, biochemical and physiological features. Turkish Journal of Biology. 2003:27:247-53.
45. Navi SS, Rajasab AH, Yang XB. *In vitro* evaluation of commercial fungicides against some of the major soil borne pathogens of soybean. Journal of Plant Pathology and Microbiology. 2016;7:340. https://doi.org/10.4172/2157-7471.1000340
46. Jabeen K, Javaid A, Ahmad E, Athar M. Antifungal compounds from Melia azedarach leaves for management of *Ascochyta rabiei*, the cause of chickpea blight. Natural product research. 2011: 25(3):264-76. https://doi.org/10.1080/14786411003754298
47. Khan MS, Ramsey MD, Scott ES. Host range studies with an Australian isolate of *Ascochyta rabiei*. Australasian Plant Pathology. 1999:28(2):170-73.
48. Bashir M, Ilyas MB. Evaluation of fungicides against ascochyta gram blight. Pakistan Journal of Botany. 1986:18:147-52.
49. Minitab IN. Minitab 17 statistical software. URL:[Computer software]. State College, PA: Minitab, Inc. (www. minitab. com), 2010.
50. Gan YT, Siddique KH, MacLeod WJ, Jayakumar P. Management options for minimizing the damage by ascochyta blight (*Ascochyta rabiei*) in chickpea (*Cicer arietinum* L.). Field Crops Research. 2006:97(2-3):121-34. https://doi.org/10.1016/j.fcr.2005.10.002