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Evaluation of Wormwood Extract as a Bird Repellent Material and Antagonistic Agent against Feces Fungi

Issa, M. A. and Hend Sh. Ghareeb
Plant Protection Research Institute, Dokki, Giza, Egypt

ABSTRACT

Wormwood extract (*Artemisia herba-alba*) was evaluate as a repellent for the house sparrow, *Passer domesticus* under laboratory and field conditions. Fungal species belonging to *P. domesticus* feces and the antifungal activity of *A. herba-alba* extract against these fungi were also investigated. The results showed that the highest concentration (10%) has the highest repellent effect with wheat consumption mean of 0.16 g and 0.15 g compared to the control (plain wheat) after four days of the laboratory and field experiments. It is worthy to mention that the lowest concentration 2.5% exhibited a high repellent effect at the first day of experiment and subsequently lost its repellent ability. Among the pathogenic fungi in feces, *Rhizopus stolonifer* (the most predominant fungus 31.7%) followed by *Fusarium subglutinans* (23.8%), *Paecilomyces variotii* (12.4%), *Cladosporium sphaerospermum* (9.2%), *Aspergillus flavus* (7.3%), *Aspergillus niger* (6%) and *Aspergillus versicolor* (5%). Whereas *Penicillium digitatum* was the least isolated fungus with occurrence 4.6%. The susceptibility of these fungi to *A. herba-alba* extract increased by increasing the extract concentration. The highest concentration 10% showed the highest antifungal activity against *A. versicolor* with mean of inhibition zone 24.3 mm. However, the lowest concentration 2.5% of this extract did not show any antifungal effects against *P. digitatum, R. stolonifer* and *A. niger*. It is concluded that wormwood is a promising biologically safe bird repellent and effective antifungal agent against pathogenic fungi of bird feces. It is economical for use than synthetic pesticides that one expensive and relatively difficult to obtain.

Keywords: Wormwood, *Passer domesticus*, repellent effect, antifungal activity.

INTRODUCTION

Birds are considered a significant threat to arable crops and a cause for concern for agriculture, its damage to the different crops is widespread (Ohuallachain and Dunne, 2013; Esther *et al.,* 2013). The house sparrow, *Passer domesticus* is one of the most important agricultural pest in Egypt. It attacks different crops such as wheat, sorghum, barley, rice, broad bean, sunflowers, pea and grapes (El-Deeb, 1991; Wilson *et al.,* 1995; Attia, 2006; Mostafa *et al.,* 2008; Abbasy *et al.,* 2012; Attia, 2013). Moreover, bird feces can contaminate food supplies, including vegetable and livestock food. It also carries large number of pathogenic fungi as *Aspergillus spp., A. niger, Cryptococcus laurentii, Trichosporon asahii, Candida albicans, C. famata, C. sphaerica, C. globosa, C. ciferri* and *Penicillium spp.* (Josiara *et al.,* 2014). Moreover, the bird feces represent a health hazard for human and animals causes many infectious diseases such as Histoplasmosis, Cryptococcus, Toxoplamosis, Ornithosis, Salmonellas, Chlamydiosis, Tuberculosis, Acariasis, Taeniasis and Trichomoniasis (Singh 1994; Singh and Walker, 1996). Most synthetic pesticides which are used to defer birds have unacceptable toxic and environmental negative impacts. The widespread use of these compounds lead to critical damage of human health and upset the environmental balance. There is an urgent need to develop safer more ecofriendly and efficient alternatives. Discovery and development of effective repellents that render crops unpalatable is of general interest (Larry and Eugeny, 1999). Natural products are excellent alternative because they can reduce harmful impacts on human health and environment (Tapondjou *et al.,* 2002) and also cost effective in countries where pesticides are expensive (Cutler, 1988; Shivanarayan and Rao, 1988). One such alternative is wormwood, *Artemisia herba-alba*, a medicinal plant used traditionally to treat diabetes mellitus and digestive system disorders in human (Haghighian *et al.,* 2008). In addition, it showed a very effective action against many parasites and worms causing serious diseases to domestic animals and also has antifeedant and toxic effects to many pests (Asta, 2016). The feeding inhibition of wormwood extract is due to its high content of phenolic acids that cause unpalatable taste to the pests (Robert *et al.,* 1984; Kamel *et al.,* 2015). On the other hand, it has a wide spectrum antifungal and antimicrobial activity against many pathogens including the following fungal species *Aspergillus nidulance, Fusarium solani* and *Pleurotus ostreatus* (Hasheminia *et al.,* 2011; Kyeong *et al.,* 1993).

The major aim of this study was to evaluate the repellent effect of wormwood, *Artemisia herba-alba* extract against the house sparrow, *Passer domesticus*. Moreover, to study its antifungal activity against fungi of the sparrow's feces.

MATERIALS AND METHODS

1. Study subjects

Thirty house sparrow, *Passer domesticus* niloticus were trapped and transported to the laboratory. This species was chosen not only because of its global distribution, but for its abundance in Egypt, and for its ability to cause large damage to the different crops. Each bird was individually caged (61× 36×41 cm) under a 12:12 light: dark cycle. All birds were given wheat seeds and tap water, after 2 weeks adaptation to the laboratory conditions 16 healthy birds were selected for use in the repellent effect of *Artemisia herba-alba* extract under laboratory conditions.

2. Stimuli

A. herba-alba leaves and stems were harvested randomly from Sinai desert, Egypt. These plant parts were washed and dried at the room temperature and then grinded to fine powder by using an electric mill. A 250 g of this powder were immersed in ethyl alcohol 95% according to the method of Mau *et al*. (2004). The obtained extract was filtered through a whatman filter paper and then freed of solvent under reduced pressure at 45ºC, using a rotary evaporator. Dried crude extract was stored at - 20ºC until used in the following experiments (Kamel *et al.,* 2015). Solutions of the repellent were prepared of concentrations 2.5, 5 and 10% poured into plastic containers containing 10 g of plain wheat seeds were vigorously mixed through by

shaking the containers for 1 min. The wheat was then laid in flat plastic trays to dry at 20-28oC for 24 h, and then the treated seeds were sealed in airtight containers until used. Plain wheat seeds (control) were also soaked in water and dried exactly as the repellent treatments (Day *et al.,* 2012).

3. Repellent effect of *A. herba-alba* extract against *P. domesticus* under laboratory conditions

During five days pretreatment period birds were presented with a cup containing 10 g of feed at 06:00 h. (down). After 2 h. the food cups were removed from the cages and the amount of feed remaining in each was weighed. During the remainder of light period the birds were free access to feed and tapwater. Feed was removed from the cages overnight so that birds were food deprived. At the end of the pretreatment period birds were divided into four groups (n = 4 / group). During the 4-day treatment period each group was fed seeds treated with wormwood, *A. herba-alba* extract at tested concentrations 2.5%, 5% and 10% for 2 h and the control group fed seeds without the extract. As in pretreatment testing occurred between 06:00 – 08.00 h. and free food was available between 0.6:00 – 0.8:00 h. Birds were food deprived overnight (Mason and Matthew 1996, Mason and Linz 1997).

4. Repellent effect of *A. herba-alba* extract against *P. domesticus* under field conditions

The repellent effect of *A. herba-alba* extract against *P. domesticus* birds at the tested concentrations 2.5, 5 and 10% which experimented in the laboratory were also tested against the same bird species in the field. This experiment was conducted in December 2016 at guava field located at El-Mesalamia village, Zagazig district, Sharkia Governorate, Egypt. Three sites were chosen in this field to carry out this experiment; plastic table was set up at each site. Each table composed of a roof 1 m^2 raised on four legs its height 1 m. Four white plastic trays containing food were placed on each table. Sparrow were trained to eat 200 g of the plain wheat which put in the trays daily for two weeks before any treatment and all the offered wheat was fully eaten daily according to Day *et al*. (2012). On each table, 10 g of plain wheat (control) were put in one of the plastic trays which placed on this table but the other three trays were contains 10 g of wheat mixed with each tested concentration of *A. herba-alba* extract. The wheat grains in the plain wheat and other tested concentrations were counted in the beginning and placed on the tables before sunrise. The remaining wheat grain in each tray was counted daily for 4 successive days after sunset and then returned to the trays for the sparrows continue feeding.

Data of the laboratory and field experiments were statistically analyzed using ANOVA test (L.S.D., $P \leq 0.05$) using SAS program (SAS institute, 1988).

5. Collection of feces samples

A total of 25 fresh samples of *P. domesticus* feces were collected from the surface of guava tree leaves in field located at El- Mesalamia village, Zagazig district, Sharkia Governorate, Egypt during the period of December 2016 to November 2017. The samples were collected in sterile bags and directly transported to the laboratory in ice box for isolation of fungi (Maysoon *et al.,* 2017).

6. Fungal isolation and identification

One gram of each feces sample was put in a test tube containing 9 ml of saline solution. All tubes were shaken for 3 min. and left for 15 min. Cultures were made from the supernatant part on potato dextrose agar (PDA) plates. Culture plates were then incubated at 28ºC for 7 days. Fungal colonies were counted and purified by sub culturing on the same culture media at 28ºC for another 7 days before characterization. Fungal isolates were identified at the Mycology laboratory, Faculty of Science, Zagazig University, Egypt according to the morphology of hyphae, spores and kind of fruiting bodies (Washinton *et al.,* 2006).

7. Antifungal activity of *A. herba-alba* extract

The antifungal activity of *A. herba-alba* extract against the fungi isolated from *P. domesticus* feces was investigated by using the disc diffusion method (Rios and Recio, 2005). The repellent concentrations of 2.5, 5 and 10% were used. All fungal isolates were cultured on PDA plates. Sterile filter paper discs (6 mm diameter) impregnated with each tested concentration of *A. herba-alba* extract and then placed in triplicate on the cultured plates. All plates were incubated at 28ºC for 7 days. The inhibition activity of each tested concentration against fungi was evaluated by measuring the diameter of the inhibition zone around the discs. Mean diameter of the inhibition zone was recorded.

Data collected from the results were analyzed using SPSS version 20 (IBM Crop., Armonk, New York). Simple means, percentages and standard error were computed as appropriate.

RESULTS AND DISCUSSION

1. Repellent effect of *Artemisia herba-alba* extract against *Passer domesticus* under laboratory conditions

Data in Table (1) and Fig. (1) show a clear variation in the consumption of *P. domesticus* to the wheat seeds treated with different concentrations of *A. herba-alba* extract and the plain wheat (control) during the experiment. *P. domesticus* birds consumed the plain wheat (control) with consumption mean of 3.65, 4.64, 3.75 and 4.22 g at 1st, 2nd, 3rd and 4th days, respectively. While, the lowest consumption was recorded at the concentration 10% of *A. herba-alba* extract with consumption mean of 0.30, 0.21, 0.13 and 0.16 g after 1st, 2nd, 3rd and 4th days, respectively. Results also indicated that with increase the tested concentration of *A. herba-alba* extract the consumption of *P. domesticus* to wheat was decrease. Analysis of data showed a highly significant difference between the tested concentrations and the control. These results are consistent with Clark (1998) reported that the natural products as plant extracts having bird repellent properties. Wormwood contains high percent of phenolic compounds which led to inhibition of the bird feeding (Robert *et al.,* 1984). Similarly, the neem extract prepared from crushed whole plants showed repellency to several avian species including Parrots (*Psittacula krameri*), House sparrows (*Passer domesticus*) and Weaver birds (*Ploceus phillippinus, Lonchura sp.*) (Rao *et al.,* 1990). Moreover, in laboratory feeding test the consumption of European Starlings to the feed which treated with aqueous extract of dried neem leaves was less than consumption of plain feed (Mason and Matthew, 1996).

Table 1. Consumption of *Passer domesticus* to wheat treated with *Artemisia herba-alba* extract concentrations under the laboratory conditions

Conc. (%)	Mean consumption of wheat (g) at indicated days			
	1st	2nd	3rd	4th
2.5	0.43	0.29	0.33	0.48
5	0.22	0.33	0.22	0.19
10	0.30	0.21	0.13	0.16
Control	3.65	4.64	3.75	4.22
P	***	***	***	***
L.S.D 0.05	0.68	1.90	0.55	0.57

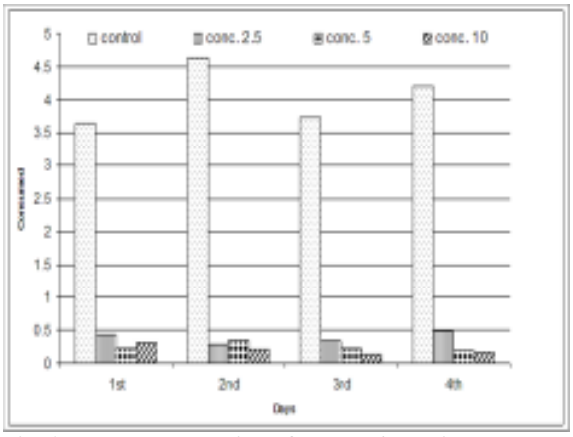

Fig. 1. Mean consumption of *Passer domesticus* to wheat treated with *Artemisia herba-alba* extract concentrations under the laboratory conditions

2. Repellent effect of *Artemisia herba-alba* extract against P. domesticus under field conditions

Repellent effect of *A. herba-alba* extract against the house sparrow, *P. domesticus* was investigated under the field conditions. As shown in Table (2) and Fig. (2) *P. domesticus* birds were highly consumed the plain wheat with consumption mean of 74.5% at the 1st day of experiment. Sparrows were completely consumed the offered plain wheat at the 2nd day with consumption 100%. *A. herba-alba* extract was effectively prevents sparrows from feeding at the different tested concentrations. The highest concentration 10% of this extract was highly reduced the consumption of sparrow to 0.86, 0.43, 0.29 and 0.15% at the 1st, 2nd, 3rd and 4th days, respectively. But the lowest concentration 2.5% was showed an observed reduction of the wheat consumption at the 1st day of experiment but after that its repellent effect was decreased till the end of experiment. That is a high significant difference in consuming wheat with the tested concentrations of *A. herba-alba* extract and control through the experiment days. Day *et al*. (2012) demonstrated that under field conditions, House sparrow, *P. domesticus* was consistently removed all the plain wheat which offered to them during the experiment, consuming 90.6 ± 5.2% of it within 1 h., wheat treated with neem oil did not effectively show repellent effect even the highest concentration. *P. domesticus* may be less sensitive to the effects of some repellents than other species.

Table 2. Consumption of *Passer domesticus* to wheat treated with *Artemisia herba-alba* extract concentrations under the field conditions

Conc. (%)	Mean consumption of wheat (%) at indicated days			
	1st	2nd	3rd	4th
2.5	3.52	35.26	52.68	68.21
5	3.94	0.79	2.5	4.06
10	0.86	0.43	0.29	0.15
Control	74.5	100	100	100
P	*	**	**	**
L.S.D 0.05	62.58	49.07	59.90	52.07

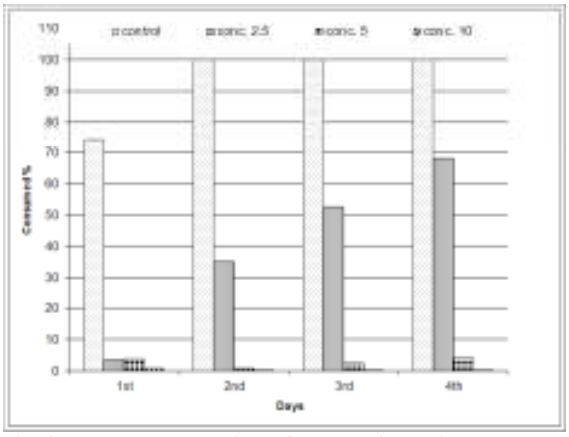

Fig. 2. Mean consumption of *Passer domesticus* to wheat treated with *Artemisia herba-alba* extract concentrations under the field conditions

Avoidance of treated feed is comparable to that devise by other avian repellents approaching within the same concentration rang (Mason and Linz, 1997). A variety of factors as feed lack, palatability and availability of alternative foods can affect avoidance of treated foods (Reidinger and Mason, 1983). Repellents often work most effectively if the treated food is novel (Greig-Smith 1987).

3. Fungal isolates from *Passer domesticus* feces

Fungal species isolated from 25 feces samples of *P. domesticus* were enumerated. The data in Table (3) shows that *Rhizopus stolonifer* was the predominant isolate (31.7%), followed by *Fusarium subglutinans* (23.8%), *Paecilomyces variotii* (12.4%), *Cladosporium sphaerospermum* (9.2%), *Aspergillus flavus* (7.3%), *Aspergillus niger* (6%), *Aspergillus versicolor* (5%) and *Penicillium digitatum* was the least isolated fungus (4.6%). Similar findings were observed by Josiara *et al*. (2014) in the feces of wild birds, they isolated various species of fungi with different percents of occurrence such as *A. niger* (4%), *Penicillium* spp. (6%) and *Mucor* sp. (4%). Several ascomycetous fungi were recovered from the feces of sparrow. *Fusarium solani* followed by *Paraconiothyrium fungicola*, *Sarocladium strictum* and *Epicoccum nigrum* (Torbati *et al.,* 2016). At the same trend, Bangert *et al*. (1988) showed that *Cryptococcus laurentii* and *Aspergillus* sp. were the most common fungal isolates from 13 fecal cultures of psittacine birds. *Aspergillus spp*. were known to be potentially infectious pathogens by characteristics as the ability to produce enzymes, adhesion capacity to the host cell, resistance to

the antifungal agent and production of hyphas which may contribute to the infectious process (Taylor *et al.,* 2005).

Its conidia cause aspergillosis which affects the respiratory system of animals. *Fusarium spp*. also causes large number of crop diseases and reported as etiologic agents of opportunistic infections in humans (Jain *et al.,* 2011). Accordingly, it is essential to remove the bird feces and eliminate or inhibit the fungi located in the feces to avoid their pathogenic effect against plants and the different living organisms.

Table 3. Fungal isolates from *Passer domesticus* feces

Fungi	No. of samples	Occurrence (%)
Aspergillus flavus	16	7.3
Aspergillus niger	13	6.0
Aspergillus versicolor	11	5.0
Cladosporium sphaerospermum	20	9.2
Fusarium subglutinans	52	23.8
Paecilomyces variotii	27	12.4
Penicillium digitatum	10	4.6
Rhizopus stolonifer	69	31.7
Total	218	100

4. Antifungal activity of *Artemisia herba-alba* extract against feces fungi of *Passer domesticus*

The susceptibility of the isolated fungi to *A. herba-alba* extract at the tested concentrations (2.5, 5 and 10%) was investigated as presented in Table (4) and Fig. (3). Results of the susceptibility test revealed that *Aspergillus versicolor* was more susceptible to *A. herba-alba* extract at the highest concentration (10%) with mean of inhibition zone (24.3 ± 2.18) than *Fusarium subglutinans* (19 ± 2.0), *Cladosporium sphaerospermum* (18.6 ± 0.32), *Aspergillus flavus* (18 ± 0.57), *Paecilomyces variotii* (16.3 ± 2.02), *Rhizopus stolonifer* (13 ± 1.52), *Penicillium digitatum* (11.6 ± 1.20) and *Aspergillus niger* (9.6 ± 0.87), respectively. Overall, *A. versicolor* was more susceptible to the all tested concentrations of *A. herba-alba* extract than the other fungi. The results also revealed that the tested extract showed varying levels of antifungal activity on the fungal isolates and as it is causing the highest inhibition against *A. versicolor* it did not record any inhibition against *A. niger* at the concentrations 2.5 and 5% and also that is no inhibition was recorded against *P. digitatum* and *R. stolonifer* at the lowest concentration 2.5%. *A. herba-alba* extract also showed an increase in antifungal activity with increase in concentration as observed in the results. The antifungal activity of wormwood against 34 species of fungi including *Fusarium solani* and *Fusarium oxysporum* has been reported by Kordali *et al*. (2005). It had a significant inhibitory activity against Sclerotinia (23.61 ± 2.12%) and *Rhizoctonia solani* (25.39 ± 0.57%) (Kamel *et al.,* 2015). Moreover, Umpierrez *et al.* (2012) demonstrated that *Artemisia absinthium* has a high antifungal effect against *Alternaria* sp. and *Botrytis cinerea.* The extract of wormwood showed severe inhibition effect against *Aspergillus nidulance*, *F. solani* and *Pleurotus ostreatus* fungi (Kyeong *et al.,* 1993). The antifungal activity of wormwood was attributed to the major component of essential oil : chamazulene which possesses a significant antifungal activity (Bozin *et al.,* 2008).

Table 4. Antifungal properties of *Artemisia herba-alba* extract against feces fungi of *Passer domesticus*

Fungal isolates	Zone of inhibition (mm / mean ± SE) at tested concentrations (%) of extract		
	2.5	5	10
Aspergillus flavus	7.0 ± 1.15	13.3 ± 1.76	18.0 ± 0.57
Aspergillus niger	No inhibition	No inhibition	9.6 ± 0.87
Aspergillus versicolor	15.3 ± 1.20	20.0 ± 2.31	24.3 ± 2.18
Cladosporium sphaerospermum	11.0 ± 1.73	15.6 ± 0.66	18.6 ± 0.32
Fusarium subglutinans	9.3 ± 2.33	13.0 ± 1.52	19.0 ± 2.0
Paecilomyces variotii	10.6 ± 1.20	14.3 ± 1.45	16.3 ± 2.02
Penicillium digitatum	No inhibition	8.0 ± 0.57	11.6 ± 1.20
Rhizopus stolonifer	No inhibition	5.0 ± 1.73	13.0 ± 1.52

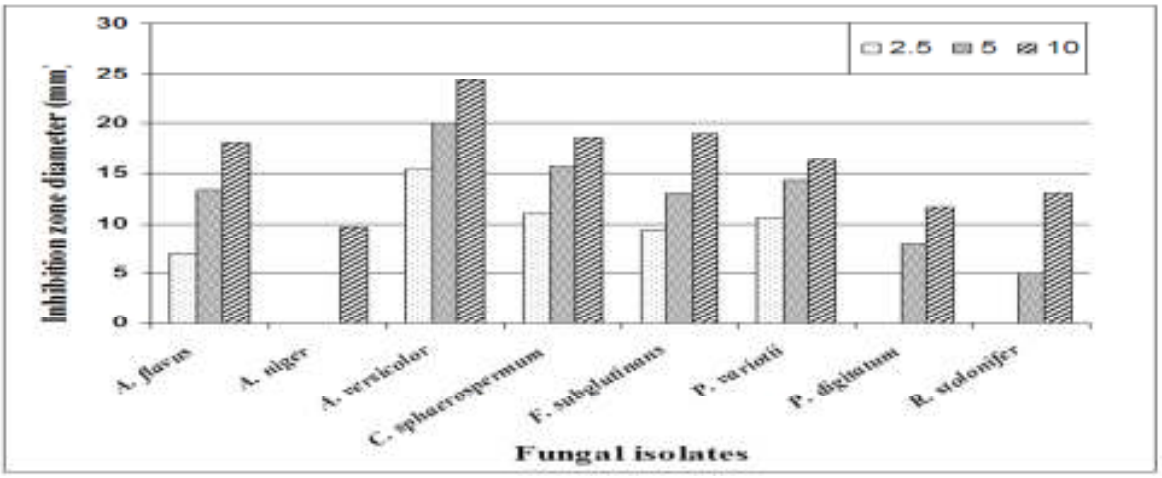

Fig. 3. Antifungal effect of *Artemisia herba-alba* extract concentrations on the growth of feces fungi

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تقييم مستخلص الشيح كماده طارده للطيور و مضاده لفطريات فضلاتها

محمد عبد الله عيسي و هند شكري غريب

معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – جيزة – مصر

تم إجراء هذه الدراسه بهدف تقييم فاعليه مستخلص الشيح عند التركيزات المختبره ٢,٥٪ و ٥٪ و ١٠٪ كماده طارده للعصفور النيل الدورى الذي يمثل أكثر أنواع الطيور انتشارا في مصر و أكثرها ضررا للمحاصيل الزراعيه. تضمنت الدراسه أيضا عزل الفطريات الموجوده في براز هذا العصفور و مدى تأثير هذا المستخلص كمضاد لهذه الفطريات عند نفس التركيزات المختبره السابقه. أوضحت النتائج أن مستخلص الشيح قد حقق أعلى تأثير طارد عند أعلى تركيز ١٠٪ بمتوسط استهلاك للقمح يعادل ٠,١٦جم مقارنه بمتوسط استهلاك٤,٢٢ جم في الكنترول (القمح الغير معامل) بعد أربعه أيام من المعامله تحت الظروف المعمليه. سجل هذا التركيز أيضا أعلى تأثير طارد في الحقل بمتوسط إستهلاك ٠,١٥٪ بالمقارنه بمتوسط إستهلاك ١٠٠ ٪ في الكنترول بعد أربعه أيام من التجربه. لقد حقق أقل تركيز من المستخلص ٢,٥٪ تأثير طارد عالي و ملحوظ عند أول يوم من التجربه و لكن بعد ذلك تناقص تأثيره الطارد في الأيام التاليه حتى نهايه التجربه. فيما يخص أنواع الفطريات الموجوده في براز العصفور النيل الدورى فقد كان فطر ريزوبس ستولونيفير هو الأكثر تواجدا في عينات البراز بينما بنسليوم ديجيتاتم هو الأقل تواجدا في العينات التي تم تجميعها. أظهر مستخلص الشيح أعلى نشاط مضاد فطري عند أعلى تركيز ١٠ ٪ ضد فطر أسبرجليس فيريكولر بينما لم يسجل أي تأثير مثبط لفطري بنسليوم ديجيتاتم و ريزوبس ستولونيفير عند أقل تركيز ٢,٥٪ و لم يثبط أيضا نمو فطر اسبرجيلس نايجر عند التركيزين ٢,٥٪ و ٥٪.