

## PATHOGENICITY OF *PHAEACREMONIUM ALEOPHILUM* ASSOCIATED WITH GRAPEVINE DECLINE IN KURDISTAN REGION-IRAQ

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### Abstract

The disease severity of *Phaeoacremonium aleophilum* on the symptomatic leaves of Taefi (cv.) ranged between 0.27-0.32 after two and four months of inoculation with significant difference from Rashmew (cv.) Pathogenicity test was performed on two cultivars, Taefi and Rashmew, under the greenhouse conditions. One year-old rooted grape cuttings were inoculated with *P. aleophilum* by two methods, injecting the spore suspension into the green shoots, and artificial inoculation of wounded shoots with mycelial mat. Symptoms appeared as brownish to black discoloration in a longitudinal section of all plant shoots. The highest canker length (20.67 mm) was produced after four months of wounding on Taefi shoots with significant difference from control treatment. Inoculation by wounding shoots was more effective than injecting shoots. *P. aleophilum* caused significant reduction in fresh and dry weight of green shoots compared with non- inoculated treatment. Under field condition, two methods of inoculation were adopted, wounding the green shoots, and drilling a hole into the grapevine arms followed by inoculation with mycelial mat. The highest canker length (17.50 mm) was obtained after 5 months on wounded shoots of Rashmew (cv.) with a significant difference from Taefi (cv.). This pathogen has been reported for the first time in Iraq.

**Key words:** *Phaeoacremonium aleophilum*, grapevine, Iraq.

### INTRODUCTION

*Phaeoacremonium* spp., have been reported as major contributors of grapevine mortality (Scheck *et al.*, 1998; and Rego *et al.*, 2000). Larignon and Dubos (1997) concluded that *P. aleophilum* and *Phaeoacremonium chlamydospora* were pioneering fungi that colonized living wood, thus preparing the wood for further colonization by the basidiomycete fungi, which were responsible for the typical decay associated with esca. Esca, also known as black measles, which is commonly associated with mature vines caused by *P. aleophilum* (Teleomorph: *Togninia minima*) as well as other species of *Phaeoacremonium* shows a range of symptoms from severe to mild or chronic. Symptoms on leaves consist of small chlorotic spots between the veins and along the margins. These spots may later coalesce and become necrotic, giving the leaves the characteristic "tiger-stripe" pattern. Shoots arising from infected wood show stunting and tip dieback; The most prominent internal symptom is the presence of brown to black spots or streaks in the xylem vessels of the woody cylinder (Scheck *et al.*, 1998; Mugnai *et al.*, 1999; Edwards *et al.*, 2001; and Gubler *et al.*, 2004).

Pathogenicity studies were recently conducted with several newly described *Phaeoacremonium* species including *P.*

*parasiticum* and *P. viticola* to determine their potential as decline pathogens (Halleen *et al.*, 2007). Sparapano *et al.* (2001) when they observed black measles (spotting on berries) on Italia (cv.) following wound inoculation of spurs and trunks of standing vines with *P. chlamydospora* and on Matilde (cv.) after inoculation of branches and spurs with *P. aleophilum*. *Phaeoacremonium* species have been isolated from grapevines from across the world.

The aim of this study was to test the pathogenicity of an isolate of *Phaeoacremonium aleophilum* associated with grapevine decline phenomena in both greenhouse and field conditions.

### MATERIALS AND METHOD

#### Isolation of pathogen.

Samples were collected from different locations of Duhok governorate. Complete vine tissues were sampled, from cane (bark and wood), bud, trunk or arm (bark and wood), leaves, clusters and roots. Plant tissue was surface-sterilized by placing in 70% ethanol for 30 s, 1% NaOCl for 1 min and again in 70% ethanol for 30 s before drying under a laminar-flow hood as described by Van Nickerk *et al.*, (2004). Small pieces of tissue were taken from the margin between necrotic and

apparently healthy tissue and plated onto 2% potato dextrose agar (PDA) with 0.25 mg/ml Chloramphenicol. Hyphae growing out from the tissue pieces were subcultured onto fresh PDA plates, and incubated at  $25\pm 2^{\circ}\text{C}$ .

#### Identification of the pathogen.

Isolates of the *Phaeoacremonium aleophilum* used in this study were identified on the bases of their morphological and molecular characterization in previous work (Haleem, 2010; Haleem *et al.* 2011).

#### Pathogenicity test in the Greenhouse.

Rooted cuttings of two *Vitis vinifera* L. cultivars, Reshmew and Taefi, were planted in pots containing 20 kg sterilized sandy loam soil (3:1) in a greenhouse at college of Agriculture, Duhok University. When they were well established and the shoots bore more than five nodes, the fourth internodes from the tip of each shoot was inoculated by *P. aleophilum*. At the time of inoculation, these internodes were green but no longer succulent (Philips, 1998). The green shoots were inoculated by using two methods: 1) Wounding shoots to 8 mm by sterilized sharp blade and a colonized agar plug about 4 mm diam. cut from the margin of seven days old culture of *P. aleophilum* was placed in the wound and covered with parafilm (Van Nickerk *et al.*, 2004). 2) Injected the green shoot by the spore suspension ( $5 \times 10^6$  spores/ml). For the control treatment the shoots were injured with no inoculation or injected with sterile water. Disease severity on the inoculated shoot estimated after 2 and 4 months depending on the grades of leaves chlorosis and necrosis in Fig (1) using a scale of four grades as show below :-

0= healthy leaf.

1= 1-25% chlorotic leaf area.

2= 26-50% chlorotic leaf area.

3= 51-75% chlorotic leaf area.

4= 76-100% chlorotic leaf area.

Disease severity was estimated according to Mickenny (1923). as follows:

$$DS = \sum d / d_{max} \times n$$

where DS is the disease severity,  $d$  is the disease rating on each plant,  $d_{max}$  is the maximum disease rating possible and  $n$  is the total number of plant examined in each replicate.

Lengths of cankers were also computed after both periods. Fresh and dry weight of green shoots and roots were recorded after 4 months of inoculation. The layout of the trail was factorial in a Complete Randomized Design (CRD) with three replications; each replicate had three plants.

#### Pathogenicity test in the field.

*P. aleophilum* was used as in the green house experiment. Inoculations were made in a vineyard on 15 yr old grapevine plants of two cultivars Reshmew and Taefi at Duhok governorate, Kurdistan region of Iraq by applying colonized agar plug cut from the margin of 7 days old culture to wounds (8 mm) on the bark, and then the inoculated wounds were wrapped with Parafilm. Inoculations were also made in mature wood by drilling a hole (4 mm wide and 1.5 cm deep) into the arm of the vine. A colonized agar plug, cut from 1 month old culture of *P. aleophilum* was placed in the wound. The wound was sealed and covered with Parafilm. The length of canker was assessed after 2.5 and 5 months (Van Nickerk *et al.*, 2004). Layout of the trial is a randomized complete block design, with three replication, 3 shoots were inoculated in each replicate. Data was statistically analyzed using SAS (SAS 1999).

## RESULTS AND DISCUSSION

#### Pathogenicity test in the Greenhouse.

Symptoms of *P. aleophilum* on the Taefi and Reshmew (cv.) leaves were observed after 35 and 45 days of inoculation respectively was a small chlorotic spots between the veins and along the margins, these spots later coalesced and became necrotic (Fig. 1A). Similar symptoms were described by (Mugnai *et al.*, 1999; Edwards *et al.*, 2001; and Feliciano *et al.*, 2004)

Inoculated shoots with the *P. aleophilum* produced dark brown canker on shoot (Fig.1 B,E) and brownish to black vascular discoloration in cross and longitudinal sections (Fig.1C,D), the typical symptoms associated with plants infected by vascular fungi (Harrington *et al.*, 2000) which might be due to the oxidation and translocation of some breakdown products of plant cells attacked by fungal enzymes (Agrios, 2005).

The disease severity of *P. aleophilum* on the symptomatic leaves of Taefi (cv.) ranged between 0.27-0.32 after two and four months of inoculation with significant difference from Reshmew (cv.) (Fig.2). The results presented in (Fig. 3) represent the interaction between pathogen and grapevine cultivars. The results showed obvious canker on young shoots of both cultivars was shown by the fungus. Based on the speed of wood colonization, Taefi cv. was rated

more susceptible to *P. aleophilum* than Rashmew cv. Inoculation of one-year old Taefi rooted cutting the canker length increased from 9.67 to 12.67 mm by wounding method and from 3.00 to 7.67 mm by injecting method after 2 and 4 month of infection by *P. aleophilum* respectively (Fig 4).

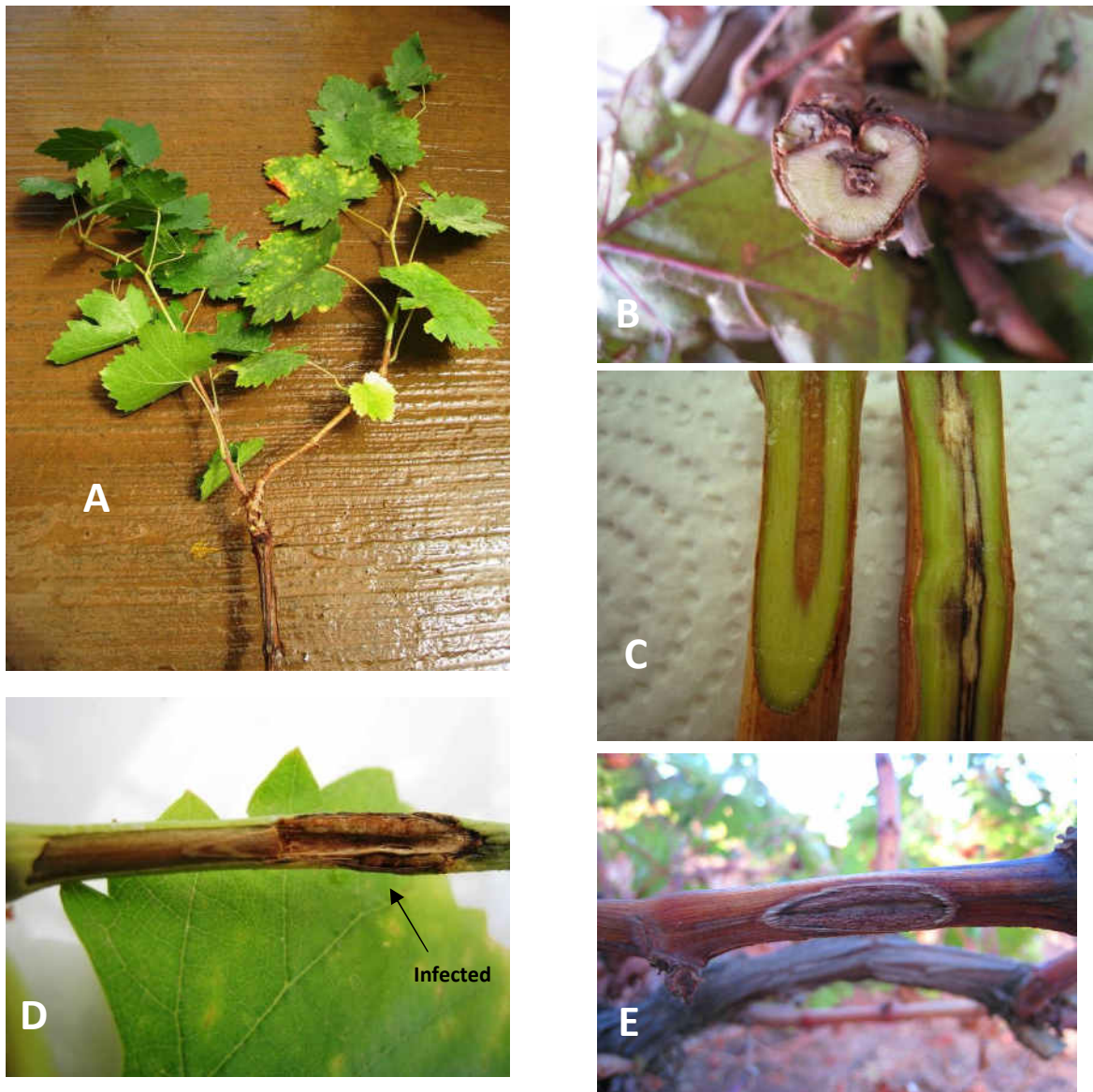
The results in Table (1) show that *P. aleophilum* decreased the fresh and dry weight of shoot and root in Taefi cultivar with significant differences from the control treatment. The study of Gubler *et al* (2001) also indicated the significant reduction in shoots infected by *Phaeoacremonium* spp.

#### **Pathogenicity test in the field.**

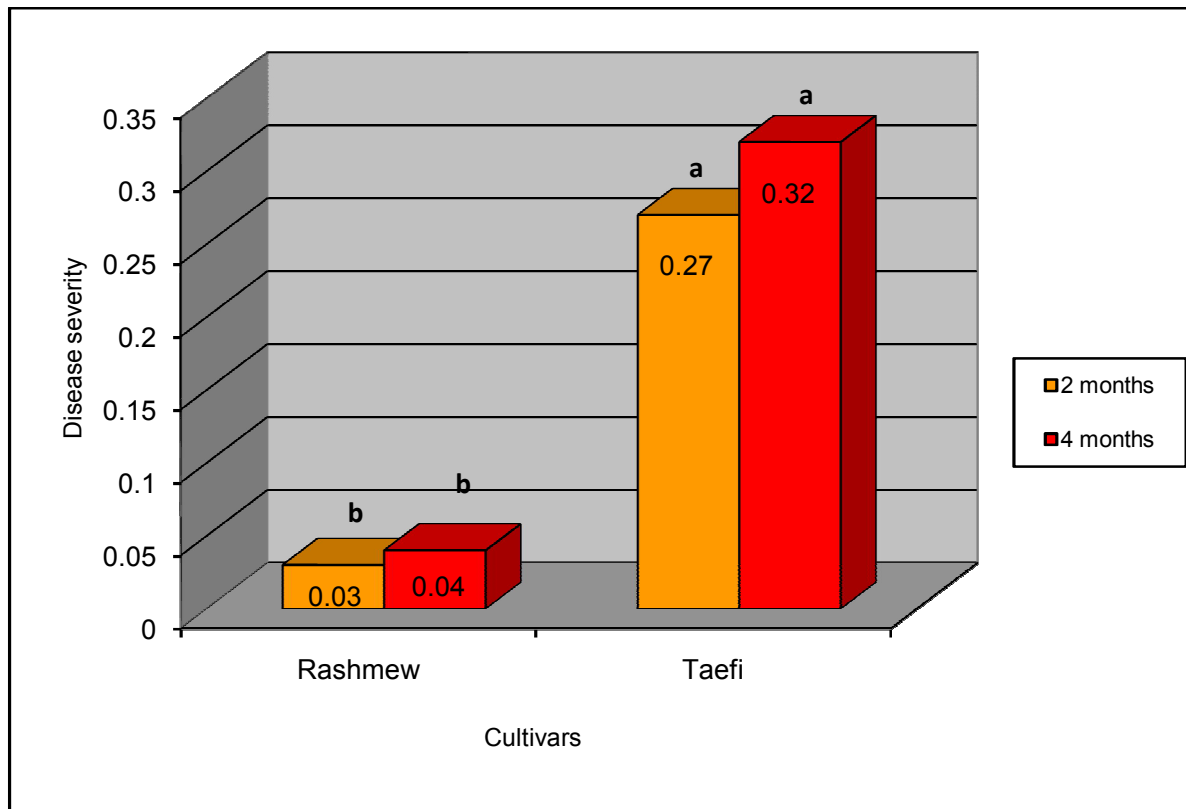
Results from the field pathogenicity test showed that the most important symptoms on the inoculated shoots with *P. aleophilum* (Fig. 1D) was vascular brownish to black discoloration in a longitudinal section of the green shoots and mature arms of a 15-years old grapevine. The

results in Table (2) shows that wounding the shoots of Rashmew (cv.) and then inoculated by the mycelium mat of *P. aleophilum* produced the highest canker length after 5 months (17.50mm). This treatment was significantly different from other treatment. Inoculation by drilling a hole in to the mature arm gave the lowest value on Taefi and Rashmew (cv.). The interaction between the pathogenic fungus and inoculation methods (Fig.5) indicated that wounding the green shoots with the mycelium of *P. aleophilum* caused a significant increasing in the canker length which reached to 13.33 mm after 5 months of inoculation.

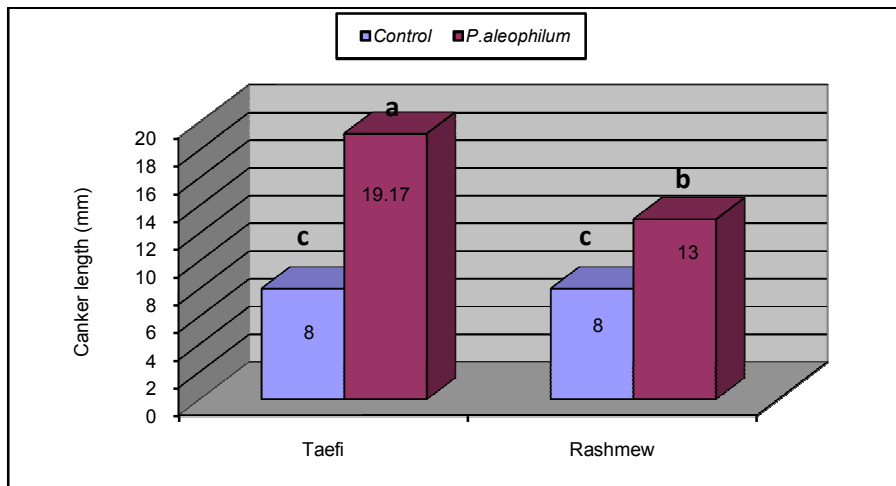
The results obtained by other researches also identified *P. aleophilum* as important grapevine pathogens causing the most severe lesions on green shoots and mature wood (Larignon and Dubos, 1997; and Gubler *et al.*, 2001).



**Fig. 1.** Profile of symptoms associated with *P. aleophilum* infected grapevine: A) Small chlorotic spots on the infected leaves. B- Dark brown, discoloration canker on young shoot inoculated by wounding under greenhouse conditions; C) Brown to black- internal discoloration visible in a cross-sectioned arms inoculated by drilling a hole, D) Brown-streaking in the longitudinal sectioned of young shoot inoculated by wounding of 15-years old Rashmew cv. under field conditions. E) Dark brown canker on young shoots wounded artificially under field conditions.



**Fig. 2.** Disease severity of *P. aleophilum* on Taefi and Rashmew cultivars after 2 and 4 months of inoculation under greenhouse conditions.



**Fig. 3.** Effect of Pathogenic fungus and Grapevine cultivars on the canker length under greenhouse condition.

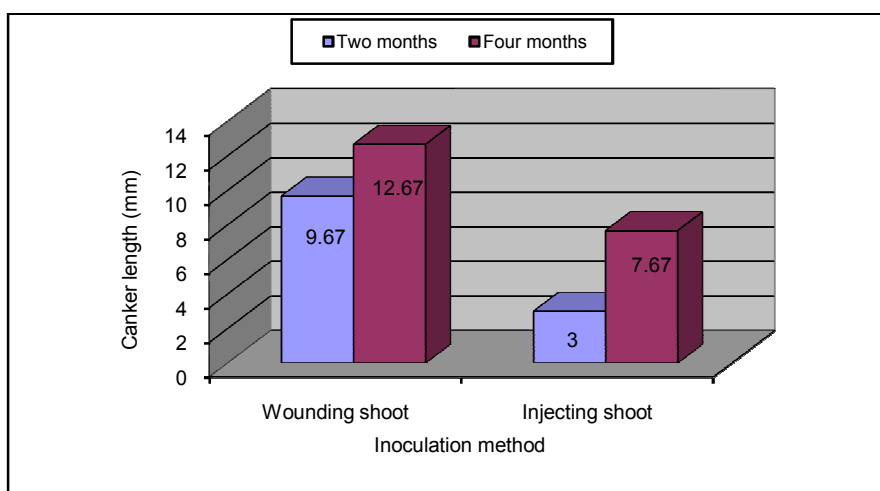


Fig 4. Effect of inoculation method on the canker length (mm) after 2 and 4 months of inoculation.

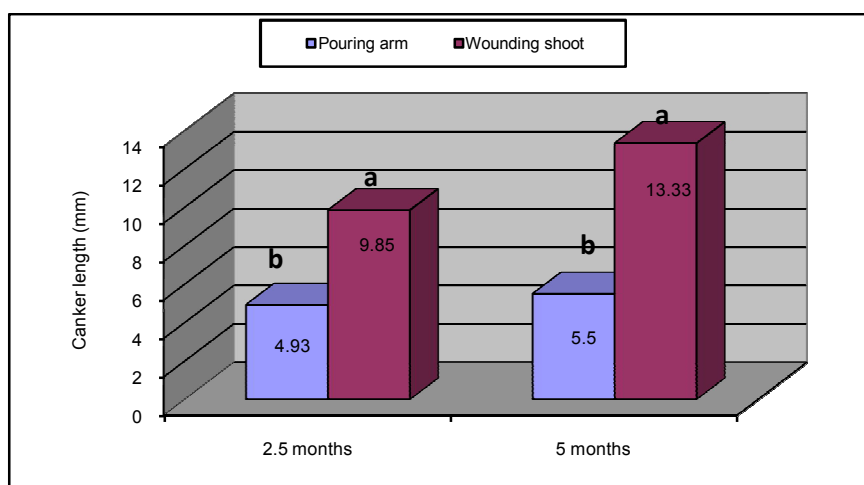


Fig. 5. Effect of inoculation method on the disease development under field condition after 2.5 and 5 months.

Table 1. Effect of isolated fungus on the shoots and root of Taefi and Rashmew cultivar.

Treatment	Shoots		Root	
	fresh weight	dry weight	fresh weight	Dry weight
	(g)	(g)	(g)	(g)
Control + Taefi c.v	42.25* b	17.88 c	18.29 b	13.45 b
Control+ Rashmew cv.	65.32 ab	25.56 bc	43.51 a	26.46 a
<i>P. aleophilum</i> + Taefi cv.	97.04 a	29.50 b	33.03 ab	19.35 ab
<i>P.aleophilum</i> + Rashmew cv.	92.82 a	41.08 a	44.83 a	24.85 a

\* Means followed by different letters are significantly different based on Duncan's Multiple Range test (P=0.05).

**Table 2.** Effect of *P. aleophilum*, grapevine cultivars and inoculation methods on the canker length (mm) after 2.5, 5 months of inoculation under field condition.

Cultivars	Inoculation method after 2.5 months		Inoculation method after 5 months	
	Wounding shoot	Pouring major arm	Wounding shoot	Pouring major arm
Taefi	8.50 *ab	4.03 c	9.17* b	4.17 b
Rashmew	11.20 a	5.83 bc	17.50 a	6.83 b

\* Means followed by different letters in each period are significantly different based on Duncan's Multiple Range test (P=0.05).

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## خلاصة

أختبرت إمراضية الفطر *Phaeoacremonium aleophilum* على سيقان صنفين من اشجار العنب (تايفي و راشمي) تحت ظروف البيت الزجاجي. تمت العدوى بطريقتين هما حقن الساق بعالق سبورى للفطر او بعمل جرح في الساق ومن ثم تلقيح مكان الجرح بقرص من الغزل الفطري. ظهر تقرح واضح على السيقان المعاملة بعد اربعة اشهر وكان اطول تقرح (20.65 ملم) على الصنف تايفي. عند عمل مقطع طولي للساق لوحظ تكون خطوط بنية الى سوداء اللون على طول الساق في منطقة التقرح. اظهرت العدوى بطريقة الجروح اكثر كفاءة من العدوى بطريقة الحقن. ادت العدوى للفطر الممرض الى اختزال الوزن الطري والجاف للساق بصورة معنوية مقارنة بمجموعة السيطرة. اما في الحقل تم عدوى النبات بقرص من الغزل الفطري بعد عمل جرح بالساق او من خلال عمل ثقب داخل الساق باستخدام ثاقب كهربائي. سجل اطول تقرح (17.5 ملم) على النبات بعد خمسة اشهر من العدوى عند استخدام الطريقة الاولى وعلى الصنف راشمي وباختلاف معنوي عن الصنف تايفي. سجل الفطر *P. aleophilum* لأول مرة في العراق.

## پوخته

تاقبكرن له سهر قهدى دوو جورى دارميوه *Phaeoacremonium aleophilum* نه خوشى كهرووى (رهش ميوو تيفي) له ژير بار و دوخى مالى شوشه. تهنينهوه به دوو ريكه روودا يه كيك به كوتانى قهد به سبورى يان به برينداربوونى قهد ئينجا كوتانى ناوجهى برينداربووه كه به دهزوولهي كهروو. سووردياريوو به شيوهيه كي روون سهر قهدى به كارهاتوو دوايى چوار مانگا دريژترين سووربووه كه (20.65ملم) سهر جورى تيفي. كاتى وهركرتنى دريژه بر كهيهك له قهد دهركهوتنى هيلى قاوهيى بو رهش به دريژايى قهد له ناوجهى سووربوو. تهنينهوه بهريكهى برينداربوون دهركهوت دواناتر به ريكهى تهنينهوهى كوتان. تهنينهوه له كهرووى تووشبووه كه به هوى كه م بوون كيشى قهدى تهر ووشك به شيوهى دياركراو. نهكه بهراورد بكريت به كوملى كونترولكراو. بهلام له كيلگه دا رووهك تووش دهبيت بههوى پهپكهيهك به دهزوولهى كهروو دواي برينداركردنى قهد يان كونكردنى قهد له ناووه به هوى كونگرى كههبايى. دريژترين سووربووه كه توماركر (17.5ملم) له سهر رووهك دواي 5 مانگه كاتى تهنينهوه به ريكهى يه كه م وله سهر جورى رهش ميوه به توماركر بو جارى يه كه م له عيراقدا. *P. aleophilum* شيوهيه كي جياواز له جورى تيفي. كهرووى