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Effect of morpho-physio chemical plant factors on preference of *Lasioderma serricorne* (f.) (Coleoptera: Anobiidae) on four cultivars of tobacco

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Abstract
Four tobacco cultivars viz. Flue-cured Virginia (FCV), Sun-cured Rustica (SCR), Dark Air-cured (DAC) and Air-cured Burley (ACB) were evaluated for preference by larvae and adults of *Lasioderma serricorne*. Leaf thickness (LT), moisture content (MC), nicotine, total sugars (TS), starch, chlorides and potassium contents were determined and the impact of these factors on beetle preference was worked out. FCV tobacco was highly preferred by both larvae (2.18) and adults (1.49) due to high percentages of MC (12.50), TS (15.84) and starch (6.89). ACB was least preferred due to low contents of MC (11.40%), TS (1.29%) and starch (1.14%). Potassium had negatively significant effect on the beetle's preference. Regression analyses revealed a positive relationship of larvae and adults with MC (0.780, 0.803) TS (0.866, 0.713) and starch (0.888, 0.765) while a negative relationship with potassium (-0.684, -0.407). LT (0.155, 0.101) nicotine (-0.047, -0.277) and chlorides (0.163, 0.305) had non-significant association with preference by both larvae and adults respectively. The relationship between MC, TS and starch is direct curvilinear as their increase had encouraged the population of *L. serricorne*.

Keywords: *Lasioderma serricorne*; preference; tobacco types; feeding, KPK, Pakistan

1. Introduction
Cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae) is a serious pest of stored tobacco [1], and is known to infest and consume all stages of the product, resulting in spoilage of at least 1% (US$300 million) of stored tobacco stocks per annum. Types of losses attributed are loss of quality and quantity of leaf tobacco, loss of value of the manufactured product at factory, wholesale and retail levels, loss in tax revenue, customer refusal and loss of goodwill [2]. The pest occurs throughout the tropical and subtropical regions of the world (Fig. 1). Although low temperature and humidity restrict its growth, yet it occurs commonly in warm buildings throughout the temperate regions [3].

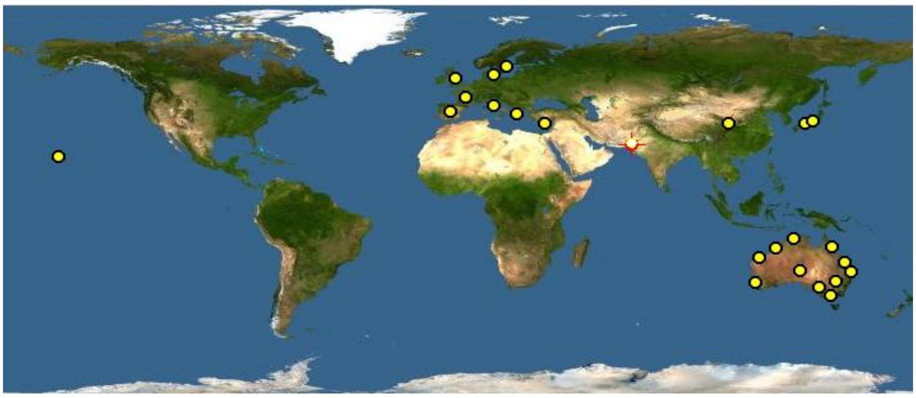

Fig 1: World-wide distribution map of *L. serricorne*
Courtesy: www.discoverlife.com

It breeds on a wide variety of commodities, including both plant and animal materials [4, 5], and is one of the several beetle pests that commonly infest warehouses and retail stores [6, 7]. Besides tobacco, it also infests a wide range of other stored commodities such as grains, rice, beans, books, furniture stuffing, nylon cord, cotton seeds, dry yeast, chilli powder, ginger, turmeric, saffron, dates, raisins, dried figs, cereals, leather, cloth, paper, cocoa, dried vegetables and even pyrethrum powder [1, 2, 8, 9]. The larvae of *L. serricorne* is the feeding stage, causing most of the damage to stored and processed tobaccos [5], adulterating the tobacco by excreta, dead beetles and production of holes in wrappers and packaging [10]. The capability of the tobacco beetle to breed on various nutrient-deficient-food-materials, like tobacco (which has relatively low contents of protein, sugars and B-vitamins, can be ascribed to the nutrient contribution made by endosymbiotic microflora (mainly *Symbiotaphrina buchneri*) in the coeca located at the junction between the foregut and midgut of *L. serricorne*. Cured leaves of tobacco also comprise several alkaloids, including nicotine, which *L. serricorne* can tolerate in some extent [11, 12]. Infestation may occur on farm storage, tobacco shipments, in the warehouses, factories and at retail outlets [8]. *L. serricorne* attacks the principal types of cigarettes, cigars, chewing and snuff tobaccos. Burley tobaccos are rarely preferred [2]. Principal cultivars of tobacco grown in Pakistan are Flue-cured Virginia (FCV), Sun-cured Rustica (SCR), Dark Air-cured (DAC), and Air-cured Burley (ACB). The type of tobacco influences the duration of the life cycle and host preference of *L. serricorne* due to various morpho-physical and chemical factors. Without knowing preference of *L. serricorne*, it is impossible to devise an effective IPM program. The variation in growth is even more remarkable when different food materials are involved. Since different host plants possess variable quantities of elements which are essentially needed for the promotion of growth and development of insects and these are not consumed and utilized equally, the extent of damage caused by polyphagous insect pests to different hosts depends on the physico-morphological characters and chemical composition of the food plants. Thus, there appear to be three mechanisms working together. Firstly, physical characters are responsible for the host selection i.e., plants having thickened cuticle of leaves are more resistant to insects [13, 14]. Similarly, moisture content also affects the extent of infestation in different plants [15]. Secondly, the host preference is shown to be related to nutritive content of the plants. Thirdly, preference of the pest for the plants is based upon factorial complex, including physical, morphological and chemical composition of the plants. The knowledge of host specificity and preference, therefore, constitutes an important field of study. Different tobacco types vary in their natural levels of chemicals. *L. serricorne* is reported to prefer high sugar, low nicotine types [16, 17], being nicotine tolerant up to 4%, but unable to survive on a diet containing above 8.25% nicotine [5]. However, high contents of nicotine influence the development, mortality and fertility of cigarette beetle while the oviposition can be increased when the substratum is previously impregnated with ethanol extracts of flue-cured tobacco [12, 18]. In Flue-cured tobacco with low nicotine and high sugars, the beetle completes its life cycle earlier i.e., in 50 days, while in tobacco with high nicotine and very small amounts of total sugars and starch, the development is very slow and the beetle completes its life cycle in 65 to 80 days. The type of tobacco also affects the oviposition i.e., lower oviposition on tobacco with higher contents of nicotine [10]. *L. serricorne* lays more eggs in flue-cured tobacco than in other tobacco types [19]. When feeding on tobacco, *L. serricorne* survives best on high sugar/low nicotine tobacco. It gives low preference to burley tobaccos [20]. A successful integrated pest management (IPM) Program cannot be implemented without knowing the feeding preferences of *L. serricorne*.

Therefore, the objective of this study was to explore the association between preference by *L. serricorne* and morpho-physio chemical profile of four tobacco types.

2. Materials and Methods

2.1 Study location and Procedure

This study was conducted in Lakson Tobacco Company Limited (an affiliate of Philip Morris International) Mardan, Khyber Pakhtoonkhwa (KPK), Pakistan. The experiments were laid out in a randomized complete block design (RCBD) with six replications.

Four types of cured tobacco viz., Flue-cured Virginia (FCV), Sun-cured Rustica (SCR), Dark Air-cured (DAC) and Air-cured Burley (ACB) were tested for preference/non-preference by the 4th instar larvae and adult stages of *L. serricorne*. Cured leaves weighing 150 g of each test plant were cleaned by conditioning (Steam) followed by Sand-reeling to remove dust. A paper sheet was spread on the floor of experimental chamber (Fig 2) measuring 45cm x 40cm x 40cm and divided into four equal sections with a 5 cm circle in center of the paper in such a way that an equal space among host plants was maintained. The clean tobacco was cut into small pieces, and 10 g of each test leaves was kept on the four sections of the paper in rearing chamber. Populations of *L. serricorne* were collected from the tobacco warehouses located in Swabi area of KPK, Pakistan. The colonies were reared on wheat flour: yeast extract (20:1) and maintained at 28±2°C and 65-70% R.H. as outlined by Ryan [8]. Colonies maintained by regular transfer of emerging adults into fresh jars having flour diet. Six female adults and larvae of *L. serricorne* obtained from the insect colonies were separately placed in center of the circle in different experiments with the objective to give them free choice of selection and feeding for twelve hours. No insecticidal measures were taken during the whole experimental period.

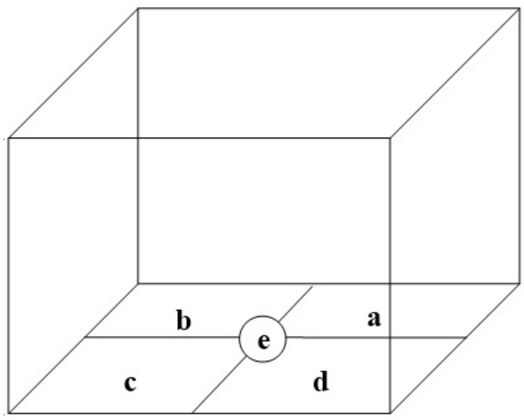

Fig 2: Experimental chamber for *L. serricorne*

(a) Flue-cured Virginia (b) Sun-cured Rustica (c) Dark Air-cured (d) Air-cured Burley (e) *L. serricorne* release Point.

2.2. Biochemical Assays

Cured leaf samples of different tobacco types, each of 200 g weight were cleaned by conditioning (Steam), Sand-reeled to remove dust, ground, passed through 1mm mesh and stored in dry polythene bags at 12 to13 % moisture content. Moisture content (%) was determined by Gravimetric Method No. 966.02 described in AOAC [21] using Brabender Moisture Meter. Thickness of lamina was determined from three different plant positions with the help of an ocular micrometer. Total sugars, nicotine and Chlorides were determined by BURKARD SCIENTIFIC SFA-2000 Method Sheet (CORESTA recommended Methods) while starch was determined by CORESTA recommended Method No. 37 described in CORESTA [22]. Potassium content was determined by Flame Photometric Method No. 966.03 described in AOAC [21].

2.3 Statistical Analyses

Analysis of Variance (ANOVA) was performed for the tobacco types preferred by the larval and adult stages of *L. serricorne* using SAS inst [23]. The impact of morpho-physical and chemical factors on preference by *L. serricorne* was worked out through simple correlation. Means were separated by Least Significant Difference (LSD) Test (Steel and Torrie, 1980) [24].

3. Results and Discussion

3.1 Rate of Preference

Significant differences were observed in the rate of preference by larval and adult stages of *L. serricorne* (Table 1). FCV tobacco was significantly preferred the most by both larval and adult stages with 2.18 and 1.49 numbers, respectively. ACB was significantly the least preferred type with minimum number of both larvae (0.64) and adults (0.82) of *L. serricorne*. SCR and DAC were preferred by statistically similar number of adults i.e., 1.25 and 1.15, respectively. These findings are in agreement with research findings of Akehurst [20] who reported that all tobaccos could be infested, lower preference given to ACB and Maryland tobacco. Ryan [8] reported that females of *L. serricorne* lay more eggs on FCV than other tobacco types and larvae are unable to survive on tobacco diet containing high contents of nicotine (8.25%). Bharati *et al.* [25] reported highest net reproductive rate (6.502) of *L. serricorne* on FCV followed by ACB (5.396). The population took 15.63 and 22.82 days to double itself on FCV and ACB, respectively. Rao *et al.* [26] reported FCV tobacco to be highly susceptible while ACB and other tobacco types were the least preferred by *L. serricorne*.

Table 1: Mean comparison of the data regarding preference by 4th instar larvae and adult stages of *L. serricorne* in four tobacco types offered as food

Tobacco type	Preference (numbers)	
	Larvae	Adults
Flue-cured Virginia	2.18a	1.49a
Sun-cured Rustica	1.58b	1.25b
Dark Air-cured	0.87c	1.15b
Air-cured Burley	0.64d	0.82c
LSD (0.05)	0.22	0.14

Means with the same letter within the column are not significantly different at 5% probability level.

Chemical composition of four tobacco types in Table 2 indicated that FCV had high percentage of moisture content (12.50), total sugars (15.84) and starch (6.89), while ACB had minimum total sugars (1.29%), moisture content (11.40%) and starch (1.14%) contents. Sun-cured rustica had the thickest (0.68 mm) leaves, maximum nicotine (4.09%) and chlorides (0.63%) contents. Potassium content was high (66.50 mg/100g) in dark air-cured tobaccos.

Table 2: Morpho-physio chemical profile of four tobacco types

Tobacco Type	Leaf Thickness	Moisture Content	Nicotine	Total Sugars	Starch	Chlorides	Potassium
	Mm	%					Mg/100 g
Flue-cured Virginia	0.29b	12.50a	1.98c	15.84a	6.56a	0.38c	40.75c
Sun-cured Rustica	0.68a	12.00b	4.09a	4.45b	6.89a	0.63a	33.50d
Dark Air-cured	0.32b	11.99b	1.42d	1.87c	2.18b	0.53b	66.50a
Air-cured Burley	0.34b	11.40c	3.25b	1.29c	1.14c	0.31c	52.75b
LSD (0.05)	0.13	0.23	0.16	1.17	0.54	0.076	0.88

Means following by different letters within the same column are significantly different from each other at 5% probability level.

Regression analyses revealed a positive relationship of larvae and adults with moisture content, total sugars and starch contents while a negative relationship with potassium (Table 3). Leaf thickness, nicotine and chlorides had non-significant effect on the preference.

Table 3: Coefficients of larvae and adult stages of *L. serricorne* with morpho-physio chemical constituents of four tobacco types

Stage interaction with	Leaf Thickness	Moisture Content	Nicotine	Total Sugars	Starch	Chlorides	Potassium
Larvae	0.155 ns	0.780**	-0.047 ns	0.866***	0.888 ***	0.163 ns	-0.684**
Adult	0.101 ns	0.803**	-0.277 ns	0.713***	0.765 ****	0.305 ns	-0.407*

ns: Non-significant , Significant at; * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.0001$

Figures 3 and 4 further elaborated the association of larvae and adults with various morpho-physio chemical constituents found in four tobacco types. The relationship between moisture content, total sugars and starch is direct curvilinear as their increase had encouraged the population of *L. serricorne*. Surani and Ashfaq [27] reported a significant and positive role of moisture content on the preference. Jakhar *et al.* [28] reported that moisture content plays an important role in the growth and development of insects. Gupta [29] and Utham *et al.* [30] found that varieties having higher moisture content are more susceptible as compared to varieties with low moisture content and these views are in accordance with our findings. Though nicotine content was statistically different in tobacco types (Table 2) yet it had a non-significant relationship with preference of larvae and adults (Table 3). Gupta *et al.* [31] tested 15 maize varieties to determine their differences in protein, starch and ash contents. The protein and ash contents were found to be positively correlated and starch negatively correlated with infestation and pest population. Potassium had significantly negative correlation with the preference by larvae and adults of *L. serricorne*. Figures 3 and 4 showed that leaf thickness, nicotine and chloride contents had a non-significant irregular relationship with beetle preference. Painter [13] claimed that thickened cuticle of the leaves is responsible for the plant resistance to insects. According to Fraenkel [14], the selection of food in polyphagous insects is based, firstly, on physical characters; and, secondly, on chemical substances such as glycosides, alkaloids and essential oils. Metha [15] found that interaction of moisture content and texture of the substrate affected the extent of infestation of different plants by the insect. Kohno *et al.* [32] reported that attractiveness of the tobacco leaves changes with change in chemical composition and concluded that active components for preference were volatile essential oils. Dowd [11] and Levinson & Levinson [12] stated that *L. serricorne* could tolerate nicotine content to some extents. Ryan [8] also reported that *L. serricorne* survives best on high sugar/low nicotine tobacco, so Flue-cured is at most risk. Carvalho [10] reported that larva of *L. serricorne* grew better on tobaccos with high sugar contents and its development was slower at high nicotine contents. She observed the highest rate of mortality on Burley (flavor) (78%) and the lowest rate on both Virginia (filler) and Burley (filler) (44%). Bharati *et al.* [25] concluded a combine effect of sugars and nicotine contents of tobacco on the life parameters of *L. serricorne*.

Hence, we conclude that Flue-cured Virginia was more favored by *L. serricorne* compared to Sun-cured Rustica, Dark Air-cured and Air-cured Burley tobaccos and appearing relatively more susceptible. Tobacco types with high contents of moisture content, total sugars and starch showed a stronger association with the larvae and adult populations of *L. serricorne*. Tobacco types with high content of nicotine and potassium were negatively correlated with the preference by larvae and adults of *L. serricorne*.

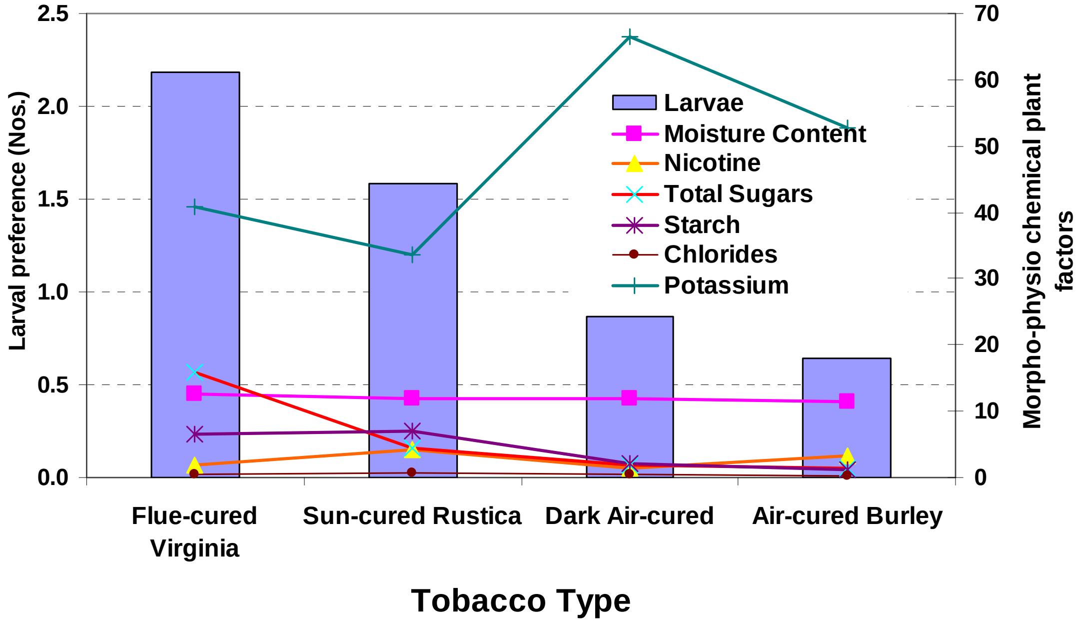

Fig 3: Relationship of larval preference with moisture content, nicotine, total sugars, starch, chlorides and potassium contents found in four tobacco types

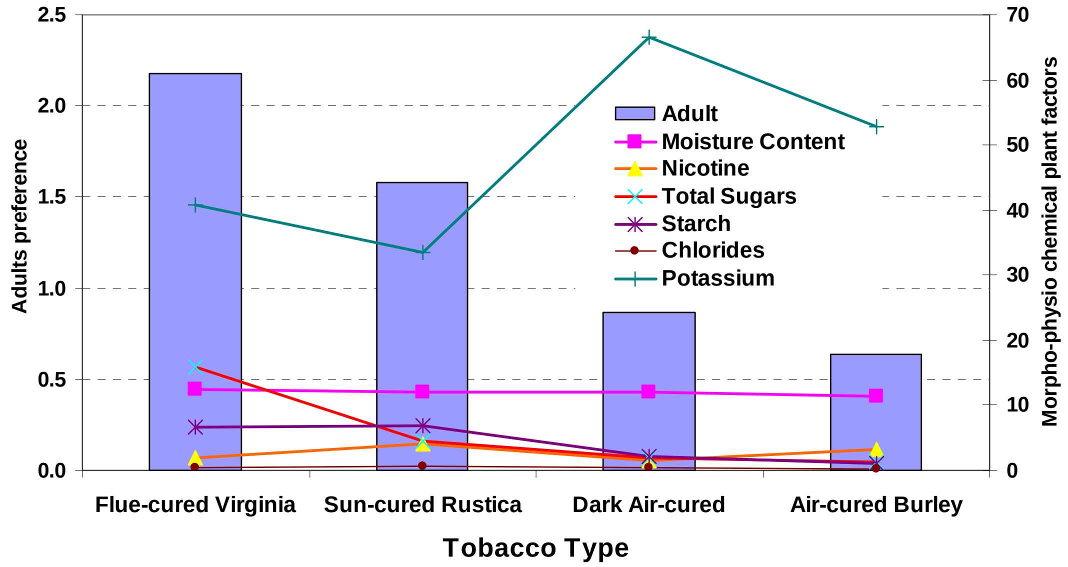

Fig 4: Relationship of adult's preference with moisture content, nicotine, total sugars, starch, chlorides and potassium contents found in four tobacco types

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