A New Approach of Split Dosage for Application of Aluminium Phosphide against the PH_3 Resistant Psocids in Stored Grain

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Summary: While inspection of wheat stores situated in districts Jehlum and Faisalabad, it was revealed that phosphine gas released from solid formulation of aluminium phosphide failed to control infestation of *Liposcelis bostrychophila* (Badonnel). Failure of fumigation as well as interception of this pest created a lot of fretfulness as the pest was not reported earlier from food godowns in Pakistan. Live presence of the pests was a proof that they survived the toxicant. Results of the godowns with fumigation failure were analyzed minutely. Survival of the pest in the treated godowns opened a window of new questions about the fate of phosphine, intensity and degree of resistance of the pest as well as status of this insect as grain pest. However, control of the pest became a top priority. This time one of the godowns was refumigated with the *Split Dosage Technique* which involved application of aluminum phosphide tablets @ 720 ppm (1 tablet/cubic meter) as first dose supplemented by 360 ppm (0.5 tablet/cubic meter) after 24, 48 hours and 240 ppm (0.33 tablet/cubic meter) each after 96, 120, and 240 hours, respectively. The other godown having the same storage capacity was fumigated with the routine practice of the Food Department, i.e., 1080 ppm (1.5 tablet/cubic meter) as first dose, supplemented by 1080 ppm (1.5 tablet/cubic meter) after 48 hours. The sealed godowns were kept closed and the results of refumigation were checked after 25 and 15 days of the initial dose administration, respectively. The technique proved successful and the infestation of psocids was completely controlled. The key to psocid survival of phosphine has been attributed to the high tolerance of their eggs. *Split Dosage Technique*, however, addressed this problem resulting in 100% control of all stages of the pest. The technique is feasible not only for Asian but also for African and other countries of the world where storage facilities are leaky and the insect pests have developed resistance against phosphine fumigant ultimately posing a threat to the food security and safety.

Introduction

Food security of the world is linked with production of food crops as well as minimizing the factors which negatively influence the produce. Among various factors, post harvest losses caused by insect pests considerably widen the gap between demand and supply of cereals. In Pakistan, stored-grains are generally invaded by traditional pests such as *Trogoderma granarium*, *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Plodia interpunctella* and *Sitotroga cerealella* with a minimum weight loss of 5-10% annually [1]. During the year 2004, entomologists working in the Punjab Food Department detected infestation of psocids *Liposcelis bostrychophila* Badonnel in wheat stores located in Districts Jehlum and Faisalabad. This was probably an exceptional and unconventional grain pest recorded in the area and the workers of the Food Department were unaware if it was harmful for stored grains? The circumstances under which the pest was detected further aggravated the problem. Incidentally, the pest was observed in those godowns which had been fumigated only few days back. Hence. their existence in the fumigated godowns was firstly attributed to failure of fumigation and leakage of gas. But detailed inspection of the godown showed that there was no sign of any gas leakage in the godowns. Consequently, appearance of psocids in the fumigated godowns was linked with their resistance to the fumigant. The problem was brought to the University of Arid Agriculture, Rawalpindi, where further investigations were made. Taxonomic studies revealed that the entire population of psocids belonged to one species, *Liposcelis bostrychophila* Badonnel. Literature relating to the pest revealed that they were not fully recognized as a pest of stored grains in the world a few years back. But during the past 10-15 years there has been a gradual world-wide recognition that psocids

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do produce a series of distinct pest problems in the area of food and grain storage [2, 3]. Cereal stores have been reported a place where they are found in abundance [4-13]. Profound surveys have further revealed that psocids are a real problem in grain stores situated particularly in hot and humid areas [14]. These tiny organisms cause considerable losses to quality of stored commodities by their existence, dead bodies and excrements as well as by their distribution of moulds. Under the new SPS agreement, presence of insects in a commodity may cause its rejection for export. Besides deterioration in quality, psocid infestation also causes quantitative losses to stored grains. They readily feed on germ and endosperm of the grains which even do not have any fungal contamination [15]. Although psocids are not included among the primary pests of stored grains, yet they are considered to be the secondary pests of the cereals [16, 17, 18]. Several publications provide information designating *L. bostrychophila* a pest of grain, but without data on weight losses [19, 20]. Some workers have, however, estimated quantitative losses caused by psocids. A weight loss of 4-5 % was recorded in rice after six months of *L. bostrychophila* infestation [21]. Similarly Pike estimated 2.9 % loss in weight of milled rice after 3.5 months of *L. paeta* infestation [7]. However, a maximum of 9.7 % loss in weight of different wheat cultivars has been reported in the Czech Republic [22, 23].

Under these circumstances, interception of a new pest may be of prime importance and gains immediate attention especially for of the researchers belonging to the developing countries, where food reserves most of the times remains at stake. That is why, psocid infestation at the provincial reserve centers of the Food Department magnetized attention of the entomologists working at the University of Arid Agriculture, Rawalpindi. Unfortunately, no meticulous information regarding control of this stored grain pest was available in the literature from Pakistan. However, they have been reported as grain pests in Bangladesh, formerly, East Pakistan [24]. The present research work was therefore carried out to find out the solution for managing this pest who has not only developed resistance against phosphine but has a potential to pose a threat for food security of our country which is already at stake.

Results and Discussion.

Relationship between Grain Moisture and Psocid infestation:

Population count of the pest in the godowns with fumigation failure indicated that it was correlated with the moisture content of the grains (Fig-1). Wheat bags having moisture percentage >14 % had relatively more number of *L. bostrychophila* adults as compared with those having moisture content below 14 %. Preference of psocids toward higher moisture contents is due to the fact that they grow and reproduce optimally at 80 % R.H [25]. It has been reported that wheat grain moisture ranging from 14 to 18% equates 80-90 % relative humidity which is considered to be ideal for the development of *L. bostrychophila* [26]. Earlier reports also confirm that ideal conditions for growth and multiplication of *L. bostrychophila* become available when relative humidity exceeds 60 % at 30^{0}C [27]. The data further revealed that wheat bags having a moisture percentage of <8 % had no or negligible infestation of *L. bostrychophila* in the same godown. This is perhaps due to the fact that low moisture content of the grains depicts low moisture availability to the harboring insects and is indirectly a measure and product of low relative humidity.

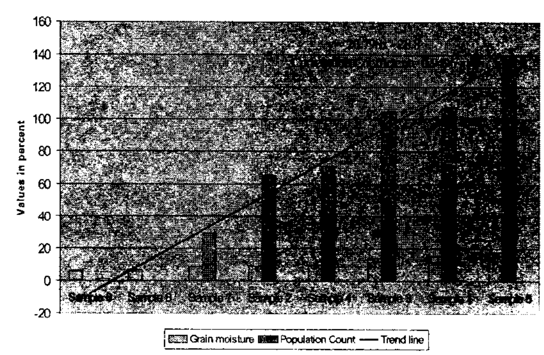

Fig.1: Trend line showing relationship between moisture content and population count of *Liposcelis bostrychophila* Badonnel at 28 ± 4 ^{0}C and 70 ± 10 % R.H.

It is reported that water makes approximately 66 % of the body mass of *L* *bostrychophila*. The adults can tolerate up to 50 % of this water below which survival becomes seriously affected. Although insect gets water from the moisture available in food as well as from the breakdown of carbohydrates, yet atmospheric moisture is the chief source of water intake for the pest [28]. When the relative humidity falls below 60 %, psocids living in grain are subjected to risk of long term desiccation because they transpire more water as they gain [29]. That is the reason we recorded no sign of psocid infestation in wheat samples having moisture contents of 5.8 and 6.23 % respectively. ANOVA pertaining to regression analysis also indicated a significant effect [F (23,202.098), P<0.000] of moisture content on population count of the pest. Grains with high moisture content indicated high degree of infestation and vice versa. The fact is also supported by the correlation coefficient (0.9496466) which has revealed a strong positive correlation between the two parameters under question. Hence, it is statistically proved that infestation of *L. bostrychophila* can be controlled just by storing the commodities in an environment of low moisture content or relative humidity.

Comparison of SDT with Routine Fumigation:

Population statistics of the pest revealed that the routine 15 days fumigation resulted in 100 percent mortality of *L. bostrychophila* adults in the check samples placed in the godown no. HT-7. On the other hand, none of the samples drawn from the wheat bags stored in different stacks in the same godown indicated 100 % mortality. Instead mortality percentage in all of the bag samples ranged from 91 to 95 % (Table-1).It was also observed that unlike check samples, the working bag samples had varying number of eggs which were missing in the samples taken from the godown HT-6 which was fumigated under *Split Dosage Technique* i.e. 25 days fumigation (Fig. 2). Statistical analysis of the fumigation results presented in Table-2 has also shown a highly significant difference between the two fumigation techniques at P<0.01 level of probability. Keeping all results in view, inference was drawn that although we achieved complete mortality of *L. bostrychophila* from the routine fumigation, yet some eggs would have survived and hatched after the end of fumigation to establish new colonies. Our findings are in line with those of Rees, who reported that in Asia some well conducted short duration fumigations are not capable of complete killing of some *Liposcelis* species, especially their eggs [30]. Besides, it has also been known that delay in hatching occurs when insect eggs are

Table-1: Split Dosage Technique for application of Aluminium phosphide tablets against *L. bostrychophila* Badonnel.

Split Dose of Phosphine (Parts per millions)	Application Time (hours)					
	0	24	48	96	120	240
Initial Dose (720 ppm)	2831 tablets	X	X	X	X	X
Second Dose (360 ppm)	X	1416 tablets	X	X	X	X
Third Dose (360 ppm)	X	X	1416 tablets	X	X	X
Fourth Dose (240 ppm)	X	X	X	944 tablets	X	X
Fifth Dose (240 ppm)	X	X	X	X	943 tablets	X
Final dose (240ppm)	X	X	X	X	X	943 tablets

Table-2: Normal schedule for application of aluminium phosphide tablets against *L. bostrychophila* Badonnel in Godown H.T. 7.

Dose	Application Time	
	0 hours	48 hours
Initial Dose (1080 pm)	4246.5 tablets	X
Final Dose (1080 pm)	X	4247.5 tablets

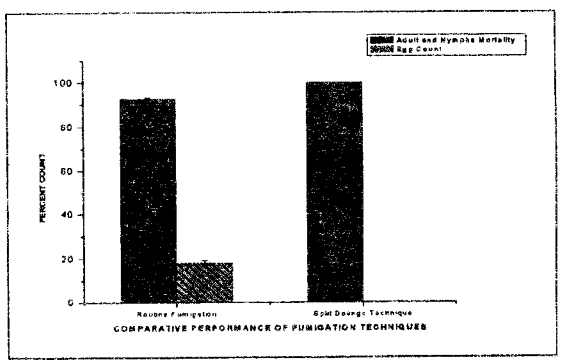

Fig. 2: Mortality percentage of Adults/ nymphs of *L. bostrychophila* Badonnel and Egg count after subjecting to regular and Split dose fumigation at 28 ± 4 ^{0}C and 70 ± 10 % R.H.

exposed to phosphine [31]. Our results are also in agreement with those of Nayak *et al.*, who attributed failure of phosphine in extinction of *L .bostrychophila* population to the delay in egg hatching [32]. They observed some delays in egg hatching ranging from 2.65 to 13.39 days at phosphine dose of 0.01 mg $Liter^{-1}$ and 1 mg $litre^{-1}$, for the susceptible and resistant strain of *L. bostrychophila*, respectively. Hence, it was concluded that fumigation failure was actually due to delayed hatching of eggs when the godowns were subjected to fumigation for the first time. Perhaps some eggs of the pest survived and hatched after completion of the fumigation to establish new colonies of more resistant adults. Results are in conformity with findings of other workers who reported that eggs of *L. bostrychophila* may survive 15 days fumigation [33].

It has been therefore suggested that fumigation protocol must comprise at least 16 days exposure at a phosphine concentration of 35 ppm or 0.05 mg $Liter^{-1}$ [32]. Some researchers have even suggested a more prolonged exposure of 28 days at $20^{o}C$ that allows all eggs to hatch to the much less tolerant nymph stage that are easily killed to yield complete control of the pest before the end of fumigation. *Split Dosage Technique* has resolved this problem as it ensures maintenance of at least 40 ppm of phosphine concentration even on the day 25 from the initial dosing. The level is sufficient to ensure complete mortality of all life stages of *L. bostrychophila.* This was the reason that the *Split dosage* exposure of adults and immature stages of *L bostrychophila* to phosphine resulted population extinction of this pest unlike the routine fumigation (Table-1). The fact is also obvious from Fig.-2 which depicted zero number of viable egg count under SDT mode of fumigation. It is also perceptible from the results of the present investigations that an exposure period of 600 hours ensured emergence of nymphs from all the quiescent eggs. The former were later on killed by the fumigant as no egg was observed in the wheat samples drawn from the godown which was subjected to split dosage fumigation technique even after the eighth week of fumigation.

T-test applied on the mean mortalities as well as egg count also revealed significant difference between the two fumigation techniques at 95 % level of confidence. Inference can be drawn from this experiment that one could avoid reinfestation of the pest at first time fumigation, simply by splitting the doses and keeping the godown sealed at least for one week more against the schedule and policy of the Food Department. But by that time it was an unresolved mystery. Anyhow, for the control of phosphine resistant *L. bostrychophila Split Dosage Technique* has proved very promising.

Experimental

Sealing of Godown:

The infested wheat in this experiment was stored in house type godown (100′x50′x20′) having storage capacity of 500.00 M.T. All ventilators as well as doors except the central one were sealed with mud plaster to prevent leakage of the gas from the godown. This procedure was done 3 days before the date of fumigation. On the date when fumigation was to be carried out, the godown was rechecked for gas leakage which usually occurs on account of sun drying. For this purpose, the only opened door was shut and the holes permitting sunlight due to the cracks in the mud plaster after three days of sun drying were plugged.

Calculation of Dose:

Refumigation of the godown was carried out by using a dose of three tablets per cubic meter. Volume of godown as well as the number of tablets required was calculated as under.

Volume = (length X width X Height) $feet^3$X 0.02831

=Cubic meters

= (100 X 50 X 20) feet ^{3} X 0.02831*

= 2831 cubic meter.

Dose = 3 $tablets\text{-}meter^{-3}$.

Total No. of tablets required = 2831X 3= 8493

or =17 flasks each having 500 tablets approx.

* 0.02831 is constant for conversion of values from cubic feet to cubic meters

Scheduling of Dose and Time for 25 days fumigation:

The godown was again prepared for carrying out refumigation. Aluminium phosphide tablets were used in split dosage according to the Table-3.

Table-3. ANOVA showing impact of Split Dosage Technique on mortality of adult/nymph as well as egg count of *L. bostrychophila* Badonnel.

Source of Variation		d.f.	S.S.	M.S.	F.C.	Sig
Adult/ Nymph mortality	Fumigation Techniques	1	623.521	623.521	124.727	.0000
	Error	46	229.958	4.999		
	Total	47	853.479			
Egg Count	Fumigation Techniques	1	3942.188	3942.188	195.279	.0000
	Error	46	928.625	20.187		
	Total	47	4870.813			

Table-4: Mortality % of *L. bostrychophila* adults and nymphs as well as egg count observed in 100 gram wheat samples randomly drawn from the stacks after subjecting normal method of fumigation and Split Dosage technique at 28±4 °C and 70 ± 10 % R.H.

Sample No.	Mean values for mortality percentage of adults/nymphs and Egg count			
	Nymphs and Adult mortality		Egg Count	
	Routine Fumigation	Split dosage technique	Routine Fumigation	Split dosage technique
1	91.3333333	100	13.6666667	0
2	94.3333333	100	25.3333333	0
3	94.6666667	100	16.6666667	0
4	92.6666667	100	29	0
5	88.6666667	100	19.6666667	0
6	92	100	15.6666667	0
7	95	100	11.3333333	0
8	93.6666667	100	13.6666667	0
T-Test Statistics: $\alpha = 0.05$	t = -9.63877517086727		t = 7.785082	

The initial dose of 720 ppm (1 tablet-meter^{-3}) was applied through the central door. The tablets were scattered on the floor all around the stacks equally. After the application of first dose, the only opened door was shut and sealed with mud to make the entire godown leak proof. Second and third dose of 360 ppm (0.5 tablets-meter^{-3}) was applied at 24 and 48 hours. Whereas, fourth, fifth and the final dose of A.P. tablets was applied through the holes made in ventilators @ 240 ppm (0.33 tablets-meter^{-3}), respectively. The holes were immediately plugged with mud after spreading the tablets in the godown. During the entire fumigation procedure, the godown was kept closed for twenty five days to maintain the concentration of phosphine for longer period. The main intention behind the longer exposure to the fumigant was not only to kill the adults and nymphs but to ensure complete hatching of the eggs which are reportedly more resistant to phosphine gas. During the entire fumigation period, average temperature remained 28± 4°C; while the relative humidity was 70± 10 %.

Scheduling of Dose and Time for 15 days fumigation:

The godown H.T.7 was also prepared for carrying out refumigation. Fumigation of this godown was undertaken in accordance with the fumigation schedule and guidelines of the Punjab Food Department. A dose of 3 tablets of aluminium phosphide was used according to the Table-4.

Initial dose of 1080 ppm (1.5 tablet-m^{-3}) was applied through the central door. Tablets were scattered on the floor all around the stacks equally. After the application of first dose, the only opened door was shut and sealed with mud to make the entire godown leak proof. The final dose of A.P. tablets was applied through the holes made in ventilators @ 1080 ppm (1.5 tablet-m^{-3}) after 48 hours of the initial dose. The holes were immediately plugged with mud after spreading the tablets in the godown, which was kept closed for 14 days after the application of last dose in accordance with the instructions of the Food Department. During the entire fumigation period the average temperature remained 28± 4 °C, whereas the relative humidity was 70± 10 %.

Counter checking the Fumigation Results:

To differentiate the phosphine susceptibility levels in adults/nymphs and eggs of the pest, fumigation results were counter checked. For this purpose eight samples (in triplicate), each weighing 100 gram of healthy wheat grains artificially infested with newly emerged nymphs as well as adults (no eggs) of *L. bostrychophila* were placed at a distance of 50 feet from each other.

Determination of Mortality Percentage:

Doors of the godowns numbering HT-7 and HT-6 were opened after twelve and fifteen days of the last dose application, respectively. After waiting for an hour, 100 gram grain samples were randomly taken from the bags positioned at different points of the stacks with the help of sampler. For the accuracy, triplicate samples from the randomly selected wheat-filled jute bags starting from lower side of the stacks, then from middle, upper and center of each stack were taken. Later on, the samples were sieved to count the number of dead and alive adults of *L. bostrychophila*. Abbot's formula was used to correct mortality percentage in the samples [34].

$$PT = \frac{PO - PC}{100 - PC} \times 100$$

Where,
PT = corrected mortality;

PO = observed mortality in treated,
PC = control mortality (all %).

$$\text{Mortality percentage} = \frac{\text{No. of dead insects}}{\text{Total no of insect in the sample}} \times 100$$

Determination of Grain Moisture:

Moisture of the grain was determined to investigate the correlation between moisture content of the grain and level of *L. bostrychophila* infestation. For this purpose, a U.S made grain moisture tester (Farmex-MT3) was used.

Conclusions

The results have helped in recommending *Split Dosage Technique* as a solution to control the infestation of resistant stored grain pests, particularly *L. bostrychophila* species. The split dosage technique is strongly recommended for the public sector warehouses which are generally leaky and poor structured. However, the protocol may not prove to be effective in all the instances of psocid infestation. Indeed it may even favour the development of a psocid problem by killing other species which could act as predators, parasites or competitors of the pest [30]. In addition, different species of *Liposcelis* have different tolerances to chemicals currently registered for structural treatments [35]. Hence, control of psocids needs an integrated approach involving species of the pest, type of fumigant used, condition of godowns, temperature as well as relative humidity. Our findings however suggest that if control of psocids is to be achieved through phosphine fumigation, the phosphine doses should be applied through *Split Dosage Technique* over an exposure time of at least three weeks to ensure complete control of all resistant as well as susceptible stages as well as species of this pest. The technique may be equally workable for all insect pests that have shown resistance to phosphine as a result of delay in egg hatching.

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