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Full Length Research Paper

Maneuvering humidity to augment response of phosphine against adult and egg stages of *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae)

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Phosphine and other fumigants have proved hazardous for treatment of stored product commodities because of their residual toxicity. In the present investigation, a comprehensive study was conducted to explore the role of different levels of humidity on efficacy of phosphine against egg and adult stages of the psocid, *Liposcelis bostrychophila* Badonnel. The results suggested that humidity play a significant role in enhancement of phosphine efficacy against the psocid, which has posed a new threat to food safety and security. It was concluded that use of low level of humidity with low doses of phosphine can be successful as an IPM strategy against the pest, which otherwise requires high humidity to survive and high level of phosphine to control. This technique may be helpful not only in reducing the phosphine doses but also in significant reduction of exposure time to achieve a specific level of mortality.

Key words: Psocid, physical control, humidity, synergism, mortality.

INTRODUCTION

Psocids have emerged as significant stored grain pests during the last few years (Rees, 1998). Out of sixteen species associated with food products, the most important seven species of the psocids include *Liposcelis bostrychophila* Badonnel*, L. entomophila* Enderlein, *L. decolor* Perman, *L. paeta* Perman, *L. divinatorius* Muller, *Lachesilla pedicularia* Linnaeus and *Trogium pulsatorium* Linnaeus. They have been found infesting stored cereals, oilseeds, pulses, spices, dried fruits, tree nuts and their processed foods (Weller and Becket, 2000; Rajendran, 1994; Anonymous, 2002). Their preferred habitats in most of the countries include granaries, warehouses, food stores, ship holds, railway boxcars, herbaria, insect collections and libraries (Broadhead, 1954; Tada, 1956; Badonnel, 1974; Turner, 1987; Sinha, 1988).

Psocids are typically found in areas of high relative humidity (r.h.) because they have trouble in controlling water loss through their exoskeletons. That is why geographical distribution of psocids include countries situated particularly in tropical climatic region such as Australia, Indonesia, Malaysia, Singapore, Philippines, Thailand, The People's Republic of China and India (Rajendran, 1994; Leong and Ho, 1995; Wang et al. 1999 and Nayak et al., 2005). Published data from these countries have designated *L. bostrychophila* as the most damaging among all psocid species infesting stored grains. Reports from the United Kingdom have annunciated common infestation of this pest in households, granaries, warehouses and food industries (Turner, 1994). Ho and Winks (1995) and Wang et al., (1999) also accounted *L. bostrychophila* as one of the dominant pest species from grain storage sector of Australia and China, respectively. No significant report is however available from South East Asian countries except those of Islam and Day (1992) as well as of Rajendran (1994) who just reported them as pests of food commodities from Bangladesh and India, correspondingly. The bio-ecological history concerning the psocid elucidates that status of this pest in South Asian and African countries needs to be re-evaluated and

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re-examined. General perception that they are only fungal predator and are not known to damage cereal grains (Ruden, 2003), is no more acceptable. This is because scientists have observed and reported their infestation on grains, which even did not have any sign of fungal infection (Kucerova, 2002). Exact status of this insect as a pest is still controversial. Literature from most of the countries has classified them as a secondary pest of the cereal grains. But Nayak et al. (1998) declared them as one of the major stored grain insect pests of Australia. Losses caused by this pest have been associated with extensive rather than intensive feeding. There had been found a strong positive correlation between economic losses and the psocid population. Investigations on feeding behaviour have indicated that they prefer to feed on grain germ rather than endosperm. They eat germ by gaining access through the seed coat cracked or mechanically damaged during harvesting, thrashing and handling or by the attack of primary stored grain pests (Nayak and Bullen, 2007). Feeding behaviour has further confirmed that this insect likes humid environment, where it feeds regularly and reproduces swiftly by obligatory thelytokous parthenogenesis. The phenomenon is responsible for immediate rebuilding of the pest population following routine fumigation of the facility.

Some workers have reported varying degrees of resistance in psocids against some fumigants such as phosphine, methyl bromide and ethylene di-bromide (Bond, 1973; Champ and Dyte, 1976; Ho and Winks, 1995; Nayak and Collins, 2003).

Routine fumigation of godowns, warehouses, granaries and food stores have proved unsuccessful in controlling this pest (Turner and Maude- Roxby, 1988). Hence, fumigation protocol and the scheduled time for phosphine fumigation that is, 7-10 days (Anon., 1985) needs to be revised. Rees (1998) has already reported failure of phosphine fumigation against this pest from Australia. Cases of phosphine resistance have also been reported from UK, Indonesia as well as from India (Pinniger, 1985; Pike, 1994 and Rajendran, 1994). Investigations on mechanism of resistance have shown that phosphine causes delay in hatching of eggs. In case of *Tribolium castaneum* Herbst, Rajendran (2000) observed significant delay when 1 - 3 day old eggs of the susceptible strain were exposed to 30 - 50 ppm phosphine for 72-120 h. Similarly, Ho and Winks (1995) reported hatching delay of 8-9 days when eggs of *L. bostrychophila* were exposed to 0.475 g m^{-3} of phosphine for 6 days. Nayak et al., (2003) have also reported a maximum delay of 13.39 days when eggs of resistant strain of *L. bostrychophila* were treated with 1 mg l^{-1} phosphine for 7 days. There are more reports showing egg survival even after 15 days exposure to phosphine (Weller and Beckett, 2000). All such reports have created uncertainty about phosphine as a tangible control measure against *L. bostrychophila*. Under the circumstances, liking for humid environment by this pest has shown a significant clue that psocid infestation may be controlled by manipulating or combining humidity factor with some appropriate fumigants (Pike, 1994). Knulle and Spadafora (1969) found that *Liposcelis* absorb water vapors from the atmosphere. They reported that sorption rate of vapors from the air above 58 per cent r.h. was found 38 times faster than that of transpiration. Below this critical equilibrium humidity (58% r.h.), rate of transpiration exceeded the sorption rate resulting in significant water loss, which lead to rapid death of the organism. Turner and Maude (1988) found that adults of *L. bostrychophila* may live without food for more than two months at 20°C provided r.h. of the room is maintained above 60%. Pike (1994) investigated tolerance of all life stages of psocids to fumigation under tropical storage conditions. All life stages were controlled when he exposed *L. entomophilus* to methyl bromide dose of 50 mg $l^{-1}h^{-1}$ at 27°C and 30% r.h. It is obvious that for each species, there is a critical r.h. below which considerable loss of water occurs from the insect body, resulting in death due to severe desiccation. The critical r.h. for different Liposcelids ranges 50-60% (Anon., 2002). It has been found that a decrease in r.h. of only 5% below the critical equilibrium may result in substantial decrease in psocid population. For example, specimens of *L. rufus* lost 50% of their water in 11 days at 25°C and 33% r.h. (Broadhead and Hobby, 1944). But most of them recovered the lost water within 6 to 7 h, flat and contracted abdomens of the dehydrated insects became inflated and returned to their original sizes and shapes when transferred to an environment having about 58% r.h. (Ebeling, 2002). Similarly, desiccated adults of *L. divinatorius*, which had become flattened and lethargic, not only recovered the lost water and became turgid within 2-3 h but started egg laying when ambient moisture was restored (Finlayson, 1932). Adults of *L. rufus* showed high mortality percentage when subjected to r.h. below 58% despite the fact they were provided with plenty of food.

Females of *L. knullei* Broadhead and Hobby transpired water twice faster as compared to *L. rufus* and died within 1 week at all humidities below their critical level.

In the same way adults of *L. bostrychophilus* survived only for 10 days and stopped egg laying when exposed to humidity below the critical equilibrium humidity (Knulle and Spadafora, 1969). These reports prove that humidity may play a significant role in proliferation or reduction of psocid population. Mashaya (2001) suggested that psocid population can be reduced by manipulating r.h. and temperature to levels below 70% and 18°C, respectively. Lin et al. (2004) also observed that *L. bostrychophila* is highly susceptible to dehydration. They recommended that population of *L. bostrychophila* causing human's nail infestation may easily be controlled by reducing r.h. of the patient's room below 60%. In countries where it is not possible to maintain humidity below the critical equilibrium humidity for longer duration, short term reduction of humidity below the critical level may enhance the efficacy of chemical and non chemical control measures.

The main objective of the research reported here was

to collect baseline data in the laboratory that will help in determination of lethal effects of different humidity levels combined with different concentrations and exposure times of phosphine gas on different stages of *L. bostrychophila*. It is hoped that findings will be helpful in formulating short term as well as long term fumigation protocol for an effective, enduring and economical control of the pest, which have otherwise shown resistance to the fumigant. The investigations would also prove promising for future investigations leading to testing of different combinations of fumigants and humidity levels against management of a variety of other stored grain pests, which had depicted some degree of resistance against the toxicants.

MATERIALS AND METHODS

Collection and culturing of test insects

Adults of *L. bostrychophila* were collected from wheat stores belonging to provincial reserves centers of the Punjab Food Department located in districts Jehlum and Tobatek Singh of the Punjab province, Pakistan. The strains were labeled as RWP-JEH and FSD-TTS, respectively. The insects were later on cultured on a diet of whole meal wheat flour, skimmed milk powder and brewer's yeast (10:10:1) at 28°C and 70% r.h. (Leong and Ho, 1995). The cultures were setup in glass bottles each having capacity of 250 ml and covered with a fine nylon cloth mesh. The jars were placed in the incubator in which saturated solution of NaCl+KCl was placed at the bottom to maintain 71.5% r.h. (Winston and Bates, 1960). The desiccators were placed in the incubators at 28 ± 1°C and 12 h: 12 h light-dark periods respectively. The insects were allowed to grow till sufficient stock colonies were established. While conducting bioassays, one to two week old insects were used for the tests. Besides, they were acclimatized at 25°C for at least 2 days before conducting the experiments (Ho and Winks, 1995).

Collection of eggs from the stock culture of *L. bostrychophila*

The eggs of *L. bostrychophila* were collected following the method used by Nayak et al. (2003). For this purpose, 500-1000 adults (2-3 weeks after maturation) of each strain were placed into separate glass jars (500 ml capacity) containing 100 g of clean wheat (14% moisture content). The jars were placed in the incubator at a temperature of 28°C. The humidity was maintained at 75% by placing saturated aqueous solution of NaCl inside the incubator. Three jars were set up for each strain.

Mouths of the jars were tightly closed by a fine nylon cloth with the help of a rubber band to avoid escaping of the adults from the jars. The jars were kept undisturbed for 48 h. Later on the grains from each jar were taken out and spread on the large Petri dishes. Keeping in view the delicacy of eggs, extra care was made to protect the eggs from mechanical injuries while collecting and using them in the experiment.

Since female of *L. bostrychophila* prefers to lay eggs in a sequence on a groove of wheat grains. This behaviour was very helpful in collection of eggs from the jars as the eggs clung to the groove tightly and escaped dislodgement during collection of the eggs. The wheat grains in each Petri dish were examined under a microscope for counting number of eggs laid by the females in the grooves. But before counting, active stages (nymphs and adults) were dislodged from the grains by exposing them to heat from a 60 W incandescent bulb placed above the Petri dish (Leong and Ho, 1990). Only eggs on the crease of the grain were used in the experiment and counted, whereas eggs laid on the other parts of the grain were dislodged. For each dose, 100 eggs on the grain were counted for three replicates. The eggs were kept at 25°C for a day before the experiments. The same procedure was repeated for control. One to 3 day old eggs were used in all the experiments.

Development of susceptible strain

The susceptible strain of *L. bostrychophila* was developed in the entomology laboratory of the University of Arid Agriculture, Rawalpindi. Originally, the insects were collected from a farmhouse granary of a remote village namely Chak Shadi of district Jehlum, Punjab province, Pakistan. The insects had never been exposed to any kind of pesticides, reportedly. The strain was labeled as LS-UAAR. To prepare an exact susceptible strain, these were reared and bred up to 22 generations at 28 ± 1°C, 71.5% R.H. While rearing, adults were fed on a medium containing whole meal wheat flour, skimmed milk powder and brewer's yeast (10:10:1) (Leong and Ho (1995). Likewise resistant strain 1-2 week old adults of the field strain were acclimatized at 25°C for at least 2 days ahead of initiating the experiments (Ho and Winks, 1995).

Generation of phosphine

Phosphine gas was generated in a fumigation chamber of 102 liter capacity developed by Ahmedani et al. (2007) by reacting known mass taken form a 3 g Aluminium phosphide tablet (Shenyang Harvest Agrochemical China; active ingredient of 1 gram phosphine/ tablet) with 5% sulphuric acid solution contained in a Petri dish. The concentration of phosphine in the fumigation chamber was determined by Bedfont EC 80 phosphine monitor. After dosing, phosphine concentrations in the fumigation chambers were checked by injecting gas sample into the monitor through a 60 ml syringe. The sample gas was circulated back into the chambers and in instances where the phosphine concentration was lower than the required, additional quantity of Aluminium phosphide was injected through a capillary tube and the concentration was rechecked to ensure the exact dose of the fumigant. Phosphine concentrations ranging 7 ppm (0.001 g^{-3}) to 1438 ppm (2.0 g^{-3}) were used in the experiment.

Tests for phosphine resistance

Resistance was tested by exposing one hundred adult insects to discriminating doses of phosphine following the FAO method (FAO, 1975). Each test was replicated thrice. A laboratory reference (susceptible) strain of the species was included in the tests to verify achievement of the discriminating doses. The data obtained so far was subjected to probit analysis (Finny, 1971). Corrected mortality was calculated as [(% mortality in treated insects percentage mortality in control insects)/ (100% mortality in control insects)] X 100 (Abbott, 1925).

Bioassy tests for adults and nymphs

The bioassay tests were conducted in glass made fumigation chamber fitted with air circulation mechanism for homogeneous distribution of the fumigant. One hundred adults (1-2 weak old after eclosion) were introduced in 200 ml glass jars having 10 g of culture medium (Leong and Ho, 1995). The jars in triplicate for each treatment were placed in the fumigation chamber. For maintaining 71.5% r.h., 200 ml of saturated solution of sodium chloride + potassium chloride contained in a dish was placed in the fumigation chamber (Winston and Bates, 1960). Room temperature was

maintained at 25 ± 1°C. The insects were tested against a series of phosphine doses vis-à-vis 0.01 mg l^{-1} (7 ppm), 0.015 (10 ppm), 0.02 (15 ppm), 0.03 (20 ppm), (0.05 (35 ppm), 0.10 (71 ppm), 0.15 (108 ppm), 0.20 (144 ppm), 0.30 (216 ppm), 0.50 (360 ppm), 1.00 (720 ppm) and 2.00 mg/l (1438 ppm). Mortality data against each dose was recorded after 6 h, 12 h, 18 h, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 14 days, 28 days and 56 days res-pectively. However, for the doses showing 100% mortality of *L. bostrychophila* adults, no more bioassay tests for the other time periods were conducted and no more daily data was recorded except 14th, 28th and 56th day as a confirmation. The same procedure was repeated for determining mortality at 53% r.h. except magnesium nitrate salt solution was used instead of sodium chloride (Bell, 1995). The control experiments at the both humidity levels were also carried out in similar way but without application of phosphine in the fumigation chambers.

Bioassy tests for eggs

The bioassay tests were conducted in glass made fumigation chamber fitted with air circulation mechanism for homogeneous distribution of fumigant. One hundred eggs (1 - 3 days old) lodged on the grain groove were placed on a black colored filter paper in the Petri dishes. The Petri dishes in triplicate for each treatment were placed in the fumigation chamber. For maintaining 71.5% r.h., 200 ml of saturated solution of sodium chloride + potassium chloride contained in a dish was placed in the fumigation chamber (Winston and Bates, 1960). Room temperature was maintained at 25 ± 1°C. The insects were tested against a series of phosphine doses vis-à-vis 0.01 mg l^{-1} (7 ppm), 0.015 (10 ppm), 0.02 (15 ppm), 0.03 (20 ppm), (0.05 (35 ppm), 0.10 (71 ppm), 0.15 (108 ppm), 0.20 (144 ppm), 0.30 (216 ppm), 0.50 (360 ppm), 1.00 (720 ppm) and 2.00 mg/l (1438 ppm). Mortality data against each dose was recorded on day 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 28 and 56. However, for the doses showing 100% hatching of *L. bostrychophila* eggs, no daily data was recorded except 14th, 28th and 56th day for re-confirmation. The same procedure was repeated for determining egg mortality at 53% r.h. except magnesium nitrate salt solution was used instead of sodium chloride (Bell, 1995). The control experiments at the both humidity levels were also carried out in a similar way but without application of phosphine in the fumigation chambers.

Critera for adult and egg mortality

The criterion for adult/nymph mortality was taken following the method described by (Ding et al. 2002; Wang et al. 2004). The adults which did not show any response and sign of movement when touched with the camel hair brush were considered as dead.

Mortality of egg was measured in terms of its hatching disability. The data was recorded up to 4 weeks after the end of fumigation and number of un-hatched eggs was considered as egg mortality.

RESULTS

Results of the present investigations (Table 1) revealed highly sig-nificant effect of phosphine on mortality *of L. bostrycho-phila* adults and nymphs ($F = 296.4791$; $df = 11,215$; $P<0.001$). Mortality response of different *L. bostrychophila* strains to phosphine as well as humidity was also found to be significant ($F = 287.9693$; $df = 2.215$ $P < 0.001$). Combined effect of phosphine and humidity on overall mortality of *L. bostrychophila* (irrespective of strains) indi-cated a strong negative correlation ($R = -0.60859$) bet-ween the phosphine concentrations and the time required to achieve 100% mortality. A maximum exposure time of 3.56 days was required at phosphine level of 0.01 mg l^{-1} followed by 2.833 and 2.11 days at 0.015 and 0.0 mg l^{-1}, respectively. However, Turkey's Multiple range test at alpha = 0.01 did not indicate any significant difference at phosphine concentrations of 0.03, 0.05, 0.10 mg l^{-1}, res-pectively. Similarly other higher phosphine levels vis-à-vis 0.20, 0.30, 0.50, 1.00 and 2.00 mg l^{-1} were also statistically similar though required lesser exposure time as compared to the all other treatments (Table 2).

As far the effect of humidity levels on mortality is concerned, it was also found significant ($F1215, 22.82 = P < 0.001$). Comparison of treatment means by Turkey's Multiple range test at alpha=0.01 has further revealed that time required for 100% mortality of *L. bostrychophila* adults/nymphs to be the maximum in case of strain FSD-TTS (5 days, followed by RWP-JEH and the susceptible strain LS-UAAR with exposure requirements of 4.33 and 2.0 days, respectively, when exposed to 0.01 mg l^{-1} phosphine at 71.5 ± 1% r.h. and 25 ± 1°C temperature (Table 4). But this time was reduced from 5.0 to 4.0 and 4.33 to 3.33 days when humidity level was reduced to 53 ± 2% r.h. under the similar conditions (Table 5). It is clear that humidity did not cause reduction in exposure time in case of LS-UAAR. The behavior of phosphine at the two humidity levels was approximately the same as mortality percentage did not increase with the proportional increase in the fumigant concentrations. Besides, phosphine concentrations 0.10 mg l^{-1} did not depict remarkable decrease in time to achieve 100% mortality. However, a remarkable decrease in time to achieve 100 percent mortality was observed when phosphine was applied at low humidity level. However, this synergism was effective at phosphine concentrations <0.03 mg l^{-1}.

Above this level, there was observed approximately similar response of phosphine at the two humidity levels. This may perhaps be due to the fact that short term exposure of the pests to lower humidity levels may not cause significant effect on the pest mortality.

In case of eggs, no significant effect of humidity levels on increasing the efficacy of phosphine against egg mortality was observed. As is evident from the (Tables 6), 100% mortality of eggs in case of LS-UAAR was observed on the day 16th at a concentration of 0.10 mg l^{-1} followed by RWP-JEH and FSD-TTS on the day 22nd and 24th respectively at phosphine levels of 1.0 and 2.0 mg l^{-1}, respectively and 71.5 ± 1% R.H and 25 ± 1°C temperatures. The results at 53 ± 2% r.h. as indicated by (Table 9) are approximately the same, which show that humidity does not play a significant role toward egg mortality. The present investigations have however revealed that phosphine because significant delay in hatching of *L. bostrychophila* eggs (Table 8 and 11). A maximum delay of 13 days was observed in case of FSD-

Table 1. Analysis of variance showing effect of different concentrations of phosphine and humidity levels on mortality of different strains *L. bostrychophila* (adults/nymphs).

SOV	Degrees of freedom	Sum of Square	Mean Square	F value	Probability
Phosphine Treatments levels (Factor-A)	11	153.816	13.983	296.4791	0.0000
Humidity Levels (Factor-B)	1	1.077	1.077	22.8282	0.0000
Interaction AXB	11	2.871	0.261	5.5343	0.0000
L. bostrychophila Strains (Factor-C)	2	27.164	13.582	287.9693	0.0000
AXC	22	34.906	1.587	33.6403	0.0000
BXC	2	0.542	0.271	5.7485	0.0040
AXBXC	22	2.708	0.123	2.6096	0.0004
Error	144	6.792	0.047		
TOTAL	215	229.875			

Table 2. Turkey's test showing comparison of mean exposure periods (days) required to achieve 100% mortalities of *L. bostrychophila* strains (adults/nymphs) in response to different concentrations of phosphine and humidity treatments at alpha = 0.010.

Mean no.	Phosphine Treatment (mg/l)	Ranked Order
1	0.01 (7ppm)	3.556 a
2	0.015 (10ppm)	2.833 b
3	0.02 (15 ppm)	2.111 c
4	0.03 (20ppm)	1.667 d
5	0.05 (35ppm)	1.667 d
6	0.10 (71ppm)	1.389 de
7	0.15 (108 ppm)	1.167 ef
8	0.20 (144 ppm)	1.000 fg
9	0.30 (216 ppm)	0.9167 fg
10	0.50 (360 ppm)	0.9167 fg
11	1.00 (720 ppm)	0.7639 g
12	2.00 (1438 ppm)	0.7639 g

TTS at phosphine level of 1.00 mg l^{-1}followed by RWP-JEH and LS-UAAR with delay of 10.5 and 4.0 days at phosphine concentrations of 0.50 and 0.05 mg l^{-1}, respectively at 71.5±1% r.h. and 25±1°C. The results have revealed that humidity did play a visible though not major role on hatchability of *L. bostrychophila* eggs as delay was enhanced from 13.0-14.0, 10.5-11.0 and from 4.0-5.5 days in case of FSD-TTS, RWP-JEH and LS-UAAR strains at the same phosphine levels at 53 ± 2% RH and 25 ± 1°C temperature.

Computation and comparison of LC50 values at two humidity levels through Probit analysis has further shown that *L. bostrychophila* strain FSD-TTS was found to be seven times more resistant as compared to the susceptible strain LS-UAAR. Similarly, the strain RWP-JEH was also found 5.14 times more resistant (Table 7 and 10).

DISCUSSION

Results of the present investigations have revealed that failure of phosphine fumigation against *L. bostrychophila* is not primarily due to the mechanism of active exclusion or detoxification system. It is mainly due to the action of phosphine on egg stage resulting in delay of egg hatching. Currently, fumigation applicators as a rule follow the FAO recommendations, which is based on 7 days exposure period for an application rate of 1.5 g m^{-3} at a temperature greater than 25°C or for 10 days at temperature greater than 15°C in gas tight enclosures (Anon., 1985). Australians follow a 7-day exposure period for phosphine fumigation including at least 1 day for the evolution of gas, usually from a metal phosphide source giving; in effect, 5-6 day fumigation. On the other hand Nayak et al. (2002) have reported that 6-7 day exposure period for phosphine dose of 1.0 g m^{-3} at 25°C have failed to control stored grain insect pests in Australia. He concluded that application of low phosphine doses to achieve complete control of *L. bostrychophila* with short term exposure is not possible. He has reported egg survival even at 2.0, 1.4 and 1.0 mg l^{-1} at 5, 6 and 7 day exposure periods respectively. It has been revealed from Tables 1 and 2 that adults and nymphs of the pest are more vulnerable to phosphine and can easily be killed by application of even low doses. During the present investigations we observed 100% mortality of adult stages within 2-5 days with a dose of 7 ppm only. The exposure period was reduced further to less than 1 day at doses >200 ppm. Nayak et al. (2002) have also reported to achieve 99.9% mortality of adults using even very low concentrations of phosphine, that is, 0.0015 mg l^{-1} and 0.0072 mg l^{-1} for susceptible and resistant strains of *L. bostrychophila*, respectively. Weller and Beckett (2000) also observed mortality of *L. bostrychophila* after 24 h of exposing the adults to phosphine concentrations ranging 10-30 ppm. However they observed survival of some nymphs and adults after 7 days. Due to the reason, an exposure period of 28 days at 25°C was recommended for 20 ppm phosphine dose.

Table 3. Turkey's test showing comparison of mean exposure periods (days) required to achieve 100% mortalities of different strains of *L. bostrychophila* (adults/nymphs) in response to different concentrations of phosphine and humidity treatments at alpha = 0.010.

Mean no.	Original Order
LS-UAAR	1.118 c
RWP-JEH	1.580 b
FSD-TTS	1.986 a

Table 4. Turkey's test showing comparison of mean exposure periods (days) and concentration of phosphine required achieving 100% mortality of 1 - 2 week old *L. bostrychophila* adults /nymphs at 25 ± 1°C and 71.5 ± 1% r.h.

Dose of phosphine mg/l (ppm)	LS-UAAR (Mean values)	RWP-JEH (Mean values)	FSD-TTS (Mean values)
0.01 (7 ppm)	2.00 a	4.33 a	5.00 a
0.015 (10 ppm)	2.00 a	3.00 b	4.66 a
0.02 (15 ppm)	1.33 b	2.00 c	3.33 b
0.03 (20 ppm)	1.00 b	2.00 c	2.00 c
0.05 (35 ppm)	1.00 b	2.00 c	2.00 c
0.10 (71 ppm)	1.00 b	1.33 cd	2.00 c
0.15 (108 ppm)	1.00 b	1.00 d	2.00 c
0.20 (144 ppm)	1.00 b	1.00 d	1.00 d
0.30 (216 ppm)	0.75 b	1.00 d	1.00 d
0.50 (360 ppm)	0.75 b	1.00 d	1.00 d
1.00 (720 ppm)	0.75 b	0.75 d	0.75 d
2.00 (1438 ppm)	0.75 b	0.75 d	0.75 d

The results have further revealed that humidity play a significant role in enhancing efficacy of phosphine against the adults and nymphs. However, implication of time factor was more pronounced than those of humidity and the phosphine concentrations. The results are in line with that of Winks (1984) who reported that exposure time of phosphine plays more important role in insect mortality than its concentration. The analogy may be equally true about the time of exposure of the insects to low humidity, which could play a significant role in case of longer exposure periods. It is also obvious that higher doses of phosphine did not yield proportional results. This is because of unique character of phosphine. Rajendran (2000) has explained that phosphine does not follow the famous Haber`s rule of (concentration) C × (time) t = (Constant) K while exerting its mortality response. Similar results were also reported by (Winks, 1986). He reported that at high pH of 3 concentrations Haber's Rule breaks down, and increased concentrations do not necessarily cause higher mortalities. At high concentrations, insects become narcotized. The time for 50% and 99% of the

Table 5. Turkey's test showing comparison of mean exposure periods (days) and concentration of Phosphine required for achieving 100% mortality of 1-2 week old *L. bostrychophila* adults /nymphs at 25 ± 1°C and 53 ± 1% r.h.

Dose of phosphine mg/l (ppm)	LS-UAAR (Mean values)	RWP-JEH (Mean values)	FSD-TTS (Mean values)
0.01 (7 ppm)	2.00 a	3.33 a	4.00 a
0.015 (10 ppm)	2.00 a	2.00 b	3.33 b
0.02 (15 ppm)	1.00 b	2.00 b	3.00 b
0.03 (20 ppm)	1.00 b	2.00 b	2.00 c
0.05 (35 ppm)	1.00 b	2.00 b	2.00 c
0.10 (71 ppm)	1.00 b	1.00 c	1.00 d
0.15 (108 ppm)	0.9167 bc	1.00 c	1.00 d
0.20 (144 ppm)	0.8333 bc	1.00 c	1.00 d
0.30 (216 ppm)	0.75 c	0.75 c	1.00 d
0.50 (360 ppm)	0.75 c	0.75 c	1.00 d
1.00 (720 ppm)	0.75 c	0.75 c	0.75 d
2.00 (1438 ppm)	0.75 c	0.75 c	0.75 d

insects to be narcotized in the concentration range 1-100 mg l^{-1} were almost co-linear with LD50 and LD99 in the range 0.01-1 mg l^{-1}, where Haber's rule held, so narcosis appeared to obey Haber's rule, even if high mortality (LD99) did not (Winks, 1973). These observations led to narcosis, along with the attendant lowered metabolic rate of narcotized insects, to be put forward as mechanisms by which insects tolerate the effect of high phosphine concentrations (Winks, 1973). Under the circumstances failure of phosphine in controlling *L. bostrychophila* reveals that problem of re-infestations in fumigated warehouses may be associated with the egg stage, which has proved more tolerant than adults and nymphs of the pest. The review of literature has revealed that duration of egg stage of *L. bostrychophila* at 20°C is on average 21 days (Weller and Beckett, 2000). Besides, Ho and Winks (1995) reported that egg stage of *L. bostrychophila* is more tolerant to phosphine as compared to adult and nymphs of this species. That is why when phosphine fumigation is carried out; fumigant arrests the egg development resulting in delay of egg hatching. The nymphs which emerge during the course of fumigations are killed whereas those emerging after the end of fumigation survive, grow up and multiply more rapidly through parthenogenesis to build up a new population once again.

Tables 3 and 4 confirms the hypothesis that phosphine appeared to have arrested the development of the egg stage. However, there are instances that resistant population of *L. bostrychophila* has survived a concentration of 2.0 g m^{-3}, 65 times greater than the susceptible strain 0.031 g m^{-3} (Nayak et al., 2002). The results regarding tolerance of egg stage are in agreement with those of Ho and Winks (1995) who reported a hatching delay of 8-9 days when eggs were exposed to 0.475 g m^{-3} of phosphine for

Table 6. Exposure time (days) and concentration of phosphine required for achieving 100% mortality in eggs of *L. bostrychophila* at 25 ± 1°C and 71.5 ± 2% r.h.

Dose of Phosphine mg/l (ppm)	Laboratory Strain LS-TSS		Rawalpindi Strain RWP-JEH		Faisalabad Strain FSD-TTS	
	Mortality %	Last day of observation*	Mortality %	Last day of Observation*	Mortality %	Last day of observation*
0.00 (0 ppm)	1.34	6 - 11**	0.67	6 - 11**	0.34	6 - 11**
0.01(7 ppm)	10.00	8 - 13	3.00	9 - 15	0.67	9 - 16
0.05 (33 ppm)	95.00	10 - 15	14.67	13 - 16	8.34	12 - 17
0.10 (71 ppm)	100.00	16 - 56	62.34	14 - 19	51.34	14 - 19
0.15 (107 ppm)	-	-	65.00	14 - 19	60.00	15 - 19
0.20 (144 ppm)	-	-	73.67	15 - 20	65.34	15 - 20
0.30 (215 ppm)	-	-	81.00	16 - 20	73.67	16 - 21
0.50 (359 ppm)	-	-	91.00	17 - 21	85.00	17 - 21
1.00 (719 ppm)	-	-	100.00	22 - 56	86.67	20 - 23
2.00 (1438 ppm)	-	-	-	-	100.00	24 - 56

*Last day of the observation was the day after which no hatching was observed even after further eight weeks
**In case of control 100% hatching of eggs took place within 11 days. No mortality was recorded.

Table 7. LC-50 and LC-99 values for egg mortality in response to phosphine exposure at 25 ± 1°C and 71.5 ± 2% r.h.

L. bostrychophila strain	LC 50 95% fudicial limit			LC 99 (95% fudicial limit			Slope
	Estimated	Lower	Upper	Estimated	Lower	Upper	
LS-UAAR	0.021	0.018	0.024	0.071	0.057	0.096	4.337600 ± 0.399735
RW-JEH	0.105	0.075	0.135	1.140	0.680	2.930	2.247395 ± 0.287692
FSD-TTS	0.139	0.093	0.193	2.392	1.191	9.072	1.883455 ± 0.265258

6 days. Previous findings on Liposcelids psocids by Rees (1994), Rajendran (1994), and Ho and Winks (1995) also confirm egg inhibition caused by phosphine. In case of egg hatching, role of humidity did not prove to be highly significant. A slight increase of two days in egg hatching period was observed at r.h. 53% as compared to that observed at 70% r.h. The results are in line with findings of Wang et al. (1999) who reported an average increase of about 1.92 day at 60% r.h. as compared to the egg development period recorded at a humidity level of 70%. However the effect of lower humidity on egg mortality and hatching period was not very much pronounced at higher doses of phosphine. This may be attributed to low humidity requirement for egg development as compared to adult and egg stages.

The other possible reason may be the higher tolerance level of egg stage to low humidity as compared to nymphal or adult stages. The present investigations reveal that irrespective of the development of phosphine resistance in several insect pests, we can still depend on the fumigant and develop effective fumigation protocols by manipulating just time of exposure even at low doses. It has also been concluded that high doses would be not able to control the pest if they are applied for short duration. Hence, the findings demand further probe by exposing mixed age cultures of *L. bostrychophila* to various exposure periods and doses of phosphine at different humidity levels to conclude a cost effective protocol leading to complete mortality of resistant strains of the pest. It is possible that mixed age culture would not be easily controlled by the doses of phosphine responsible for adult mortality alone. It is expected that humidity would play a key role in reducing time for population extinction in case of long term fumigation protocols.

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Table 8. Effect of different concentrations of phosphine inducing hatching delay of *L. bostrychophila* eggs at 25 ± 1°C and 71.5 ± 2% r.h.

Dose of phosphine mg/l (ppm)	Susceptible strain LS-UAAR				Rawalpindi strain RWP-JEH				Faisalabad strain FSD-JHA			
	Hatch%	Range of hatching period (days)	Mean hatching period (days)	Delay (days)	Hatch%	Range of hatching period (days)	Mean hatching period (days)	Delay (days)	Hatch%	Range of hatching period (days)	Mean hatching period (days)	Delay (days)
0.00 (0 ppm)	100	6 - 11	8.5	0.00	100	6 - 11	8.5	0.00	100	6 - 11	8.5	0.00
0.01 (7 ppm)	90	8 - 13	10.5	2.0	100	9 - 15	12.0	3.50	100	9 - 16	12.5	4.00
0.05 (33 ppm)	5	10 - 15	12.5	4.0	85.33	13 -16	14.5	6.00	93.33	12 - 17	14.5	6.00
0.10 (71 ppm)	0	16 - 56		-	37.66	14 - 19	16.5	8.00	48.66	14 - 19	16.5	8.00
0.15 (107 ppm)	-	-	-	-	35	14 - 19	16.5	8.00	40	15 - 19	17.0	8.50
0.20 (144 ppm)	-	-	-	-	26.33	15 - 20	17.5	9.00	34.66	15 - 20	17.5	9.00
0.30 (215 ppm)	-	-	-	-	19	16 - 20	18.0	9.50	26.33	16 - 21	18.5	10.00
0.50 (359 ppm)	-	-	-	-	9	17 - 21	19.0	10.5	15	17 - 21	19.0	10.50
1.00 (719 ppm)	-	-	-	-	0	22 - 56			13.33	20 - 23	21.5	13.00
2.00 (1438 ppm)	-	-	-	-	0	0			0	24 - 56		

Table 9. Exposure time (days) and concentration of phosphine required for achieving 100% mortality in eggs of *Liposcelis bostrychophila* at 25 ± 1°C and 53 ± 2% r.h.

Dose of phosphine mg/l (ppm)	Laboratory strain LS-TSS		Rawalpindi strain RWP-JEH		Faisalabad strain FSD-TTS	
	Mortality %	Last day of Observation*	Mortality %	Last day of Observation*	Mortality %	Last day of observation*
0.00 (0 ppm)	1.00	6 - 11**	1.34	6 - 11**	2.34	6 - 11**
0.01(7 ppm)	9.67	8 - 13	1.67	9 - 16	2.67	9 - 16
0.05 (33 ppm)	95.00	12 - 16	14.00	12 - 16	7.00	13 - 18
0.10 (71 ppm)	100.00	17 - 56	60.00	14 - 19	51.67	14 - 19
0.15 (107 ppm)	-	-	68.34	15 - 19	60.67	15 - 19
0.20 (144 ppm)	-	-	72.00	15 - 20	67.00	15 - 20
0.30 (215 ppm)	-	-	81.34	17 - 20	72.67	17 - 20
0.50 (359 ppm)	-	-	93.00	18 - 21	84.67	18 - 21
1.00 (719 ppm)	-	-	100.00	22 - 56	89.00	20 - 23
2.00 (1438 ppm)	-	-	-	-	100.00	24 - 56

*Last day of the observation was the day after which no hatching was observed even after eight weeks.
**In case of control 100% hatching of eggs took place within 11 days. No mortality was recorded.

Table 10. LC-50 and LC-99 values of egg mortality in response to phosphine exposure at 25 ± 1°C and 53 ± 2% r.h.

L. bostrychophila strain	LC 50 95% fudicial limit			LC 99 (95% fudicial limit			Slope
	Estimated	Lower	Upper	Estimated	Lower	Upper	
LS-UAAR	0.021	0.018	0.024	0.071	0.056	0.096	4.340889 ± 0.394410
RW-JEH	0.108	0.081	0.135	0.989	0.625	2.211	2.418401 ± 0.290530
FSD-TTS	0.140	0.090	0.194	2.262	1.138	9.008	1.925742 ± 0.290281

Table 11. Effect of different concentrations of phosphine inducing hatching delay *of L. bostrychophila* eggs 25 ± 1°C and 53 ± 2% r.h.

Dose of phosphine mg/l (ppm)	Susceptible Strain LS-UAAR				Rawalpindi Strain RWP-JEH				Faisalabad Strain FSD-TTS			
	Hatch %	Range of hatching period (days)	Mean hatching Period (days)	Delay (days)	Hatch%	Range of hatching period (days)	Mean hatching period (days)	Delay (days)	Hatch%	Range of hatching period (days)	Mean hatching period (days)	Delay (days)
0.00 (0 ppm)	100.00	6-11	8.50	0.00	100.00	6 - 11	8.50	0.00	100.00	6-11	8.50	0.00
0.01 (7 ppm)	90.00	8-13	10.50	2.00	100.00	9 - 16	12.50	4.00	100.00	9-16	12.50	4.00
0.05 (33 ppm)	5.00	12-16	14.00	5.5	86.00	12 - 16	14.00	5.5	93.00	13 - 18	15.50	7.00
0.10 (71 ppm)	0.00	17-56	-	-	40.00	14 - 19	16.50	8.00	48.33	14 - 19	16.50	8.00
0.15 (107 ppm)	-	-	-	-	31.66	15 - 19	17.00	8.50	39.33	15 - 19	17.00	8.50
0.20 (144 ppm)	-	-	-	-	28.00	15 - 20	17.50	9.00	33.00	15 - 20	17.50	9.00
0.30 (215 ppm)	-	-	-	-	18.66	17 - 20	18.50	10.00	27.33	17 - 20	18.50	10.00
0.50 (359 ppm)	-	-	-	-	7.00	18 - 21	19.50	11.00	15.33	18 - 21	19.50	11.00
1.00 (719 ppm)	-	-	-	-	0.00	22 - 56	-	-	11.00	20 - 23	22.50	14.00
2.00 (1438 ppm)	-	-	-	-	-	-	-	-	0.00	24 - 56	-	-

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